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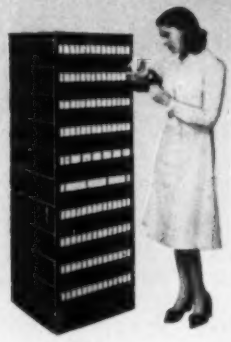

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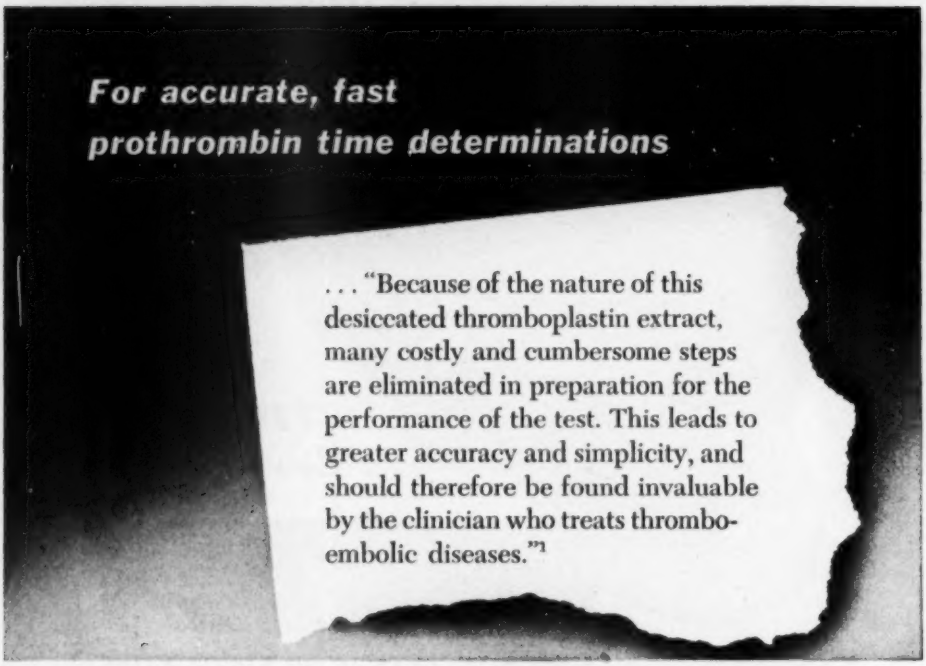
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2. J. M. Soc. New Jersey 48:1 (Jan.) 1951.

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DISSECTING ANEURYSMS OF THE AORTA IN PERSONS UNDER FORTY YEARS OF AGE

IRA GORE, M.D.
WASHINGTON, D. C.

CERTAIN characteristics of the dissecting aneurysm of the aorta observed in persons under 40 years of age deserve emphasis, since they shed some light on the pathogenesis of this vascular condition. In a study of the pathologic features in 72 cases of dissecting aneurysm from the Armed Forces Institute of Pathology [1] the medial degeneration underlying the actual dissection was seen to be of two types. The first involved the elastica primarily; the second, the aortic musculature. The first type was more often observed in young persons; the second in patients over 40 years of age. A combination of damage and depletion of both types was present in the aortas from an intermediate age group, although lesions of the two types did not necessarily occur in the same foci.

Dissecting aneurysm of the aorta is generally regarded as a disease of the old, although it has often been reported in younger persons. Five hundred eighty cases gathered from the medical literature previous to 1943 by Schnitker and Bayer [2] included 141 (24%) in which the affected person was less than 40 years of age.

The ages of the patients whose records are filed at the Armed Forces Institute of Pathology differ from those generally quoted for dissecting aneurysm of the aorta because young men predominate in the army population, from which the bulk of this material was drawn.[1] The age of the patients in 32 of 85 fatal cases (38%) was less than 40 years at the time of death. The ages of these 32 patients by decade are listed in Table 1, which also records the histologic type of medial disease in each instance. It is to be noted that in the majority of these cases the aorta showed a degenerative process affecting primarily the elastic lamellae of the media. By contrast, dissecting aneurysm in the aorta of persons over 40 was characterized by a degenerative process of muscle, most often alone and less frequently associated with lesions of the elastica.[1]

Such of the clinical and pathologic data as lend themselves to tabulation are appended in Table 2.

From the Armed Forces Institute of Pathology.

Dr. Gore is now with the Veterans Administration Hospital, Fort Douglas, and the Department of Pathology, Utah University College of Medicine, Salt Lake City.

1. Gore, I., and Seiwert, V. J.: Dissecting Aneurysm of the Aorta: Pathologic Aspects; An Analysis of 85 Fatal Cases, A. M. A. Arch. Path. **53**:121-141, 1952.

2. Schnitker, M. A., and Bayer, C. A.: Dissecting Aneurysm of the Aorta in Young Individuals, Partly in Association with Pregnancy, with Report of a Case, Ann. Int. Med. **20**:486-511, 1944.

TABLE 1.—*Dissecting Aneurysms of the Aorta in Persons Under Forty: Histologic Type of Medial Disease by Decade*

Age	Primarily Elastic	Primarily Muscle	Types Coexistent	Origin Unknown	Total Cases
Under 20	1	..	..	..	1
20-29	13	2	2	1	18
30-39	2	3	4	4	13
Total	16	5	6	5	32
Per cent	50.0	15.5	19.0	15.5	100

TABLE 2.—*Clinical, Anatomic, and Histologic Data from Thirty-Two Cases of Dissecting Aortic Aneurysm in Patients Under Forty* *

Case No.	AFIP Acc. No.	Age	Hypertension	Aortic Sclerosis	Medial Vascularization	Congenital Anomalies	Duration of Illness	Sclerosis of Adventitial Vasa	Adventitial Reaction
					Elastic Type of Medial Degeneration				
1	38366	19	—	—	++	.....	1½ d.	—	Early
2	205405	20	—	—	+	.....	Chronic	—	Productive
3	509	22	—	++	++	.....	Acute	—	Early
4	106973	22	—	—	+	Arachnodactyly, coarctation	16 mo.	—	Productive
5	89517	23	—	—	++	Bicuspid a. v.†	Acute	—	None
6	97254	24	—	—	+	Malformed tricuspid valve	2 wk.	—	Productive
7	118106	24	±‡	—	++	.....	1 d.	—	Early
8	77210	25	—	—	+++	.....	7 mo.	+ —	Late, productive
9	201601	25	—	—	++	Bicuspid a. v., widely patent foramen ovale	Acute	—	Late, productive
10	124046	26	—	—	++	Bicuspid a. v., aberrant left renal artery	Acute	—	None
11	206262	26	—	++	++	.....	Acute	—	Early
12	107549	27	—	++	+++	Arachnodactyly	2 d.	—	Early
13	170441	27	—	++	++	Widely patent foramen ovale, Meckel's diverticulum	5 d.	—	Productive
14	125348	27	—	—	++	Bicuspid a. v.	Acute	—	Early
15	138190	31	—	—	+	.....	Acute	—	Productive
16	139038	35	—	+++	++	Bicuspid a. v., absent right coronary artery	2½ d.	—	Early
					Combined Type of Medial Degeneration				
17	95745	23	+	++	+++	.....	28 d.	+	Productive
18	184463	24	—	—	+	Arachnodactyly	Acute	—	None
19	160866	32	—	+	±+	.....	5 d.	—	Productive
20	164042	36	+‡	+	++	Coarctation, accessory spleen	Acute	±	Early
21	164752	38	—	++	++	Bicuspid, a. v.	2 d.	—	Early
22	102153	39	—	++	++	Coarctation	Acute	—	Productive
					Muscle Type of Medial Degeneration				
23	74657	25	—	—	++	Bicuspid a. v., coarctation	19 d.	—	Productive
24	112351	29	—	+	+	Bicuspid a. v.	2½ d.	..	Inadequate specimen
25	142351	33	—	—	+	.....	6 d.	—	Productive
26	109592	34	—	+	++	Patent ductus arteriosus	5 d.	—	Early
27	51601	38	+	++	++	.....	6 d.	—	Productive
					Unknown Type of Degeneration (No Histologic Studies)				
28	110973	20	—	—	....	Bicuspid a. v.	7 d.	..	.....
29	135867	36	—	....	....	Polycystic kidneys	Acute	..	.....
30	135684	36	—	+	....	.....	1 d.	..	.....
31	60766	37	+	+++	....	.....	1 hr.	..	.....
32	80049	39	—	+	....	.....	16 mo.	..	.....

* Key to symbols: — indicates none; + indicates the presence of a particular feature, from questionable (±) to very marked (+++).
† Aortic valve.
‡ Systolic only.

PATHOLOGIC OBSERVATIONS

The initial site of intimal rupture, the occurrence of multiple tears (Cases 9 and 26), the extent of intramural dissection, and the frequency of reentry or rechannelization were not significantly different in the various age groups. Twenty-five, or 77%, of the intimal lacerations were observed in the ascending aorta, a significantly greater incidence than the 53% for similarly located lesions among 53 persons over 40 years of age. Transverse orientation of the intimal tear, which was most frequent in the older age group, was not observed when medial degeneration was predominantly of the elastic type. Of the tears adequately described, 50% were longitudinal, in contrast to 14% similarly oriented in patients over 40. The tear was in the transverse portion of the arch in three of the remaining cases, in the distal portion of the arch (just beyond the origin of the left subclavian artery or the insertion of the ligamentum arteriosum) in two, and in the thoracic aorta in two. In one case (Case 18) dissection had involved the renal artery immediately distal to its origin.

Intimal tears were absent in two cases. One (Case 28) was that of a 26-year-old white man who died of hemopericardium one week after the onset of severe substernal pain. The second (Case 17) deserves special comment. Postmortem examination disclosed an isolated hemorrhage, 12 cm. in length, splitting the thoracic and abdominal portions of the aortic media; there was no communication with either the intima or the adventitia. The lesion represented a coincidental occurrence, intramural "apoplexy," with hypertensive encephalopathy the cause of death. Similar examples of isolated medial hemorrhage have been reported elsewhere [3] and constitute the chief evidence for the suggestion that intimal laceration in dissecting aneurysm may be secondary to extravasation into the media. The dissecting aneurysm in another case (Case 27) was also a coincidental finding in fatal hypertensive cardiorenal disease, but here there was an intimal tear. In two other cases, both examples of chronic dissection, the aneurysm failed to rupture externally (Cases 2 and 32). The external hemorrhages, occasionally multiple, in the remaining 27 cases were distributed as follows: pericardium, 22; thorax, 4; mediastinum, 3, and retroperitoneum, 2.

The location of the intimal laceration provided no index of the extent of the basic medial degeneration. A better clue was furnished by the length of the dissection tract. It is well established and generally accepted, however, that foci of medial disease remote from the path of the dissecting hemorrhage may easily be demonstrated. For that matter, it is not uncommon to find similar foci in the aorta when death has resulted from diverse and unrelated causes. Medial degeneration in one case (Case 27) was so advanced that mere manipulation sufficed to split the media into outer and inner portions throughout the aorta to its bifurcation, although the actual tract of the hemorrhage did not extend beyond the arch.

Histologic distinctions between so-called spontaneous rupture and dissecting aneurysm of the aorta were not observed. Clinically, however, the former process proved to be more promptly fatal. As indicated in Table 3, there were 13 instances of spontaneous rupture. Nine of the patients died within one day, and only one lived longer than five days (Case 23).

3. Gore, I.: Pathogenesis of Dissecting Aneurysm of the Aorta, A. M. A. Arch. Path. **53**:142-153, 1952.

By contrast, only 6 of the 19 patients who did not have spontaneous ruptures died in less than one day. Two patients lived for two and four weeks, respectively (Cases 6 and 17), and four others survived the initial symptoms for months (Cases

TABLE 3.—*Extent of Dissection in Thirty-Two Cases of Dissecting Aneurysm in Persons Under Forty Years of Age*

No appreciable dissection: "spontaneous" rupture	13
From proximal aortic segment to or beyond the bifurcation	7
From proximal aortic segment to the abdominal aorta	5
From proximal aortic segment to the innominate, left carotid, or left subclavian artery	5
From proximal aortic segment to the beginning of the thoracic aorta	2
Total	32

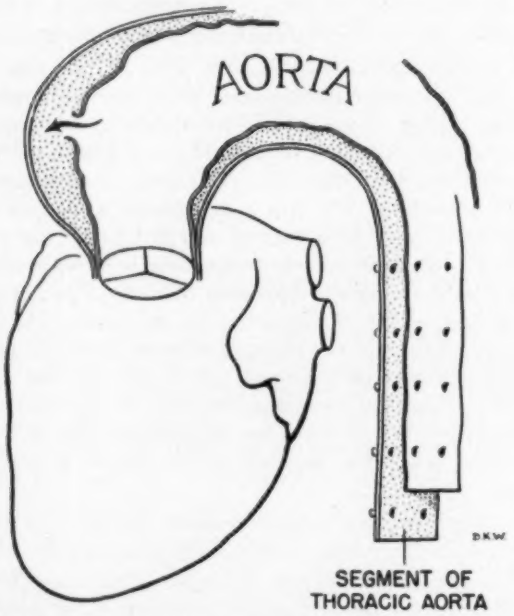

Fig. 1 (Case 20).—Path of a dissecting aneurysm within the wall of the aorta. An intimal tear in the dilated proximal aorta admits blood from the circulation. Observe the transected segmental vessels in the thoracic aorta, which may permit reentry of blood into the natural channel.

2, 4, 8, and 32). The histologic type of aortic degeneration subsequently observed in these cases could not be correlated with a greater or lesser likelihood of prolonged survival.

Rechannelization or reentry of the intramural tract into the aortic lumen was observed in each of four examples of chronic dissecting aneurysm. Since only two of the patients had died of hemorrhage, rechannelization appears to offer some protection against that eventuality. In two cases (Cases 14 and 15) intimal tears at the distal limit of the dissection channel did not suffice to prevent death from cardiac tamponade 12 hours and less than 2 hours, respectively, after the onset of

symptoms. Depending upon pressure relations, reentry of blood may occur through the natural ostia of segmental vessels which have been torn or divided intramurally by the dissecting hemorrhage (Fig. 1). With lengthy dissections particularly, therefore, it seems certain that functional circulatory reentry must be much more frequent than would be indicated by the incidence of intimal breaks at the distal end of the false channel.

CLINICAL CONSIDERATIONS

There is little point in reviewing the detailed symptomatology of the patients in the younger age group, since it was identical with that in the older groups. The onset was abrupt, painful, and often shocking; there was the same shifting and migration of pain. Bizarre and confusing symptoms resulted from impairment of circulation to the head, extremities, spinal cord, or viscera, and consequently the problem of differential diagnosis was no less complex. Two unusual cases in which death resulted from rupture of a huge aneurysm of the ascending aorta deserve

TABLE 4.—*Aortic Aneurysms Preceding Rupture of Dissecting Hemorrhage*

Case No.	AFIP Acc. No.	Age at Death, Yr.	Associated Congenital Anomaly	Type of Medial Degeneration	Interval Between Induction into Army and Death
5	89517	23	Bicuspid aortic valve	Elastic	9 mo.
9	201601	25	Bicuspid aortic valve	Elastic	4 6/12 yr.
10	124946	26	Bicuspid aortic valve	Elastic	4 9/12 yr.
28	116973	26	Bicuspid aortic valve	Unknown	2 4/12 yr.
14	125348	27	Bicuspid aortic valve	Elastic	2 9/12 yr.
20	164042	30	Slight aortic coaretation	Elastic and muscle	.....
22	102153	39	Slight aortic coaretation	Elastic and muscle	7 mo.

special mention. In each instance the clinical record listed symptoms attributable to the presence and growth of the aortic deformity. There were four other cases (Table 4) in which similar lesions developed silently, and a seventh case for which clinical information was lacking. One case (Case 9) was that of a white man who died at the age of 25 years, 22 months after leaving military service. During his 32 months in uniform he had complained rather nebulously of lack of well-being. A 15-month interval following his return to civilian status was featured by three episodes of chills and fever, each precipitated by strenuous labor. Four months before death enlargement of the heart was recognized clinically. At autopsy, there was an enormous aneurysm of the ascending aorta, which had ruptured into the right atrium. The pericardial space was entirely obliterated, and therefore the febrile episodes observed during life might be interpreted as the result of acute pericardial irritation. Since there was definite roentgenologic evidence of an expanding aneurysm during life, it is tempting to consider that the relation of the febrile attacks to effort pointed to a leakage of blood as the cause of the pericardial reaction. The heart was considerably enlarged, for, in addition to the adhesive pericarditis, there was sufficient distortion of the aortic valve by the aneurysm to have resulted in gross insufficiency. The second patient (Case 14), a white man of 27, had suffered from shortness of breath and substernal pain on severe exertion

for two years. The symptoms were attributed to chronic valvulitis because of a short systolic and a long transmitted diastolic murmur audible over the aortic area. However, autopsy disclosed considerable dilatation produced by an aneurysm, fully accounting for insufficiency and dysfunction of th aortic valve. In this case, incidentally, there was moderate fibromyxomatous thickening of the distal portions of the bicuspid aortic leaflets. Similar productive changes noted by Edwards and associates [4] in the aortic wall at the site of impingement of the blood-stream jet distal to the stenosis in coarctation suggest that these changes in the valve may have been produced by a like mechanism. Identical valve lesions in Case 2 of Tobin, Bay, and Humphreys [5] were ascribed to congenital malformation and, to judge from the gross description, seem also to have been present in Gloggengiesser's [6] case.

The pathologic evidence in four cases of the present series indicated a dissecting aneurysm of considerable duration. Yet in one (Case 2) there was only an 11-day history of congestive heart failure. Repeated bouts of cardiac decompensation over a period of several months were reported in the other three; in one of them (Case 32) death occurred in such an episode, in the other two (Cases 4 and 8) death followed rupture of the aneurysm. Episodes of pain which might have suggested the nature of the process were recorded only in the last case; in the others the basis for the functional inadequacy of the heart was a clinical mystery. For that matter, it is difficult to account for the occurrence of heart failure except in one instance of aortic insufficiency resulting from distortion of the valve ring and dislocation of the unaltered cusps (Case 32). Aortic regurgitation produced in this fashion has previously been reported.[7] It does not seem reasonable to attach significance to the relatively minor increase in blood volume that results from the presence of the false channel. Cardiac hypertrophy was present in each of the four cases.

ROLE OF HYPERTENSION AND STRAIN IN DISSECTING ANEURYSM

Hypertension and strain have been considered augmenting or precipitating rather than primary etiologic factors in the genesis of dissecting aneurysm. Repeated assertions of their primary role in the pathogenesis of dissecting aneurysm of the aorta ignore instances unassociated with elevated arterial pressure or physical exertion. Certainly the presence of hypertension in only 3 of the 32 cases discussed here is inconsistent with its acceptance as an etiologic factor. In two additional cases only the systolic pressure was elevated. The presence of a degenerative process in the aortic media in every case must, therefore, be regarded as the *conditio sine*

4. Edwards, J. E.; Christensen, N. A.; Clagett, O. T., and McDonald, J. R.: Pathologic Considerations in Coarctation of the Aorta, Proc. Staff Meet., Mayo Clin. **23**:324-332, 1948.

5. Tobin, J. R., Jr.; Bay, E. B., and Humphreys, E. M.: Marfan's Syndrome in the Adult: Dissecting Aneurysm of the Aorta Associated with Arachnodactyly, Arch. Int. Med. **80**:475-490, 1947.

6. Gloggengiesser, W.: Sogennante Spontanruptur der Aorta bei Isthmusstenose und Medianekrosen der Aorta, Frankfurt. Ztschr. Path. **56**:11-22, 1941.

7. Resnik, W. H., and Keefer, C. S.: Dissecting Aneurysm with Signs of Aortic Insufficiency: Report of a Case in Which the Aortic Valves Were Normal, J. A. M. A. **85**:422-424, 1925. Roberts, J. T.: Medionecrosis Aortae Idiopathica Cystica: Report of Case, with "Healed" Dissecting Aneurysm, Am. Heart J. **18**:188-200, 1939. Peery, T. M.: Incomplete Rupture of the Aorta: A Heretofore Unrecognized Stage of Dissecting Aneurysm and a Cause of Cardiac Pain and Cardiac Murmurs, Arch. Int. Med. **70**:689-713, 1942.

qua non of the disease. It seems paradoxical, perhaps, that the ineffective nature of the repair renders such lesions vulnerable to the sequence which leads to dissection. As part of the inadequate tissue reaction new-formed vasa appear, having thin walls supported only by the structurally deficient tissue of the diseased media. It is the rupture of such vessels intramurally which initiates the hemorrhagic dissection.[5] Elevated blood pressure, either as part of a systemic disease or as a consequence of strain or emotion, is to be regarded as an augmenting, not an essential, factor.

CONGENITAL ANOMALIES ASSOCIATED WITH DISSECTING ANEURYSMS

Rather striking was the high incidence of congenital anomalies, frequently multiple and usually vascular, 18 of the 32 patients being so affected. Deformities were present in a greater proportion of the younger persons, occurring in 11 of the 17 aged 20 to 29, but in only 6 of the 13 in the subsequent decade. Developmental anomalies were noted in 10 of 16 patients in whom medial disease was of the elastic type, and the remaining 8 occurred in an approximately equal proportion of patients with degenerative changes in the medial musculature and in those with muscular and elastic lesions.

Frequent references to the association of deficiencies of the aortic wall with congenital vascular deformities are found in the literature. Two examples of congenital malformation of the arteries were included among the 80 cases collected by Peacock[8] in 1863. Holle[9] reported multiple aortic aneurysmal dilatations in a 3-month-old infant and postulated congenital mesodermal inadequacy as the basis for the underlying elastic-tissue defect in the media. In Bronson and Sutherland's[10] case, a boy of 5 years with coarctation of the aorta died when a fusiform aneurysm of the ascending portion ruptured into the pericardium. In von Rokitansky's[11] case, dissecting aneurysm in a boy of 8 was associated with congenital narrowing of the aorta, a ventricular septal defect, and distortion of the pulmonary artery. Wasastjerna[12] described considerable dilatation of the ascending aorta and coarctation complicating dissecting aneurysm in a boy of 13. Similar malformations, but with an unruptured intramural dissecting hemorrhage, in a boy of 14 years were reported by Hamilton and Abbott.[13] Elastic fibers were completely absent from the media of an aneurysm proximal to a complete stenosis of the descending aorta. Symptoms

8. Peacock, T. B.: Report on Cases of Dissecting Aneurysm, Tr. Path. Soc. London **14**: 87-99, 1963.

9. Holle, G.: Über mehrfache angeborene mesenchymale Defektbildungen mit "Medionecrosis aortae" bei einem 3 monate alten Säugling, Ztschr. ges. Inn. Med. **2**:502-510, 1947.

10. Bronson, E., and Sutherland, G. A.: Ruptured Aortic Aneurysm in Childhood, with the Report of a Case, Brit. J. Child. Dis. **15**:241-258, 1918.

11. von Rokitansky, K.: Über einige der wichtigsten Krankheiten der Arterien, Denkschr. Akad. Wissensch. **4**:41, 1852.

12. Wasastjerna, E.: Ein Fall von Aortaruptur nach Schlittschuhlaufen bei einem scheinbar gesunden 13 jährigen Knaben, Ztschr. klin. Med. **49**:405-410, 1903.

13. Hamilton, W. F., and Abbott, M. E.: Coarctation of the Aorta of the Adult Type: I. Complete Obliteration of the Descending Arch at Insertion of the Ductus in a Boy of 14; Bicuspid Aortic Valve; Impending Rupture of the Aorta; Cerebral Death; Statistical Study and Historical Retrospect of 200 Recorded Cases, with Autopsy, of Stenosis or Obliteration of Descending Arch in Subjects Above Age of 2 Years, Am. Heart J. **3**:381-421, 1928.

of dissecting aneurysm in an athletic white man, whose case was reported by Hall,[14] were first noted when he was 17. Fifteen years later he died of aortic insufficiency and congestive heart failure; at autopsy, a patent ductus arteriosus was found. Clinical evidence of aortic coarctation and dissecting hemorrhage observed by Kinney, Sylvester, and Levine[15] in a 23-year-old white woman, six and a half months pregnant, was verified post mortem. Microscopically, the aortic media showed cystic degeneration and considerable depletion of elastic tissue. Harrison[16] described extensive medial degeneration in a 27-year-old patient with severe coarctation. Yettra and Lasky[17] reported aneurysmal dilatation of the aorta as the result of cystic medial degeneration in a white man of 29 years. A soldier 36 years of age, who died of rupture of the ascending aorta, was found by Gloggengiesser[6] to have an aneurysmal dilatation of the proximal segment, coarctation, and a congenital bicuspid deformity of the aortic valve. Microscopically, the aortic wall proximal to the stenosis showed mucocystic degeneration. Though coarctation was absent, Milne and co-authors[18] described similar deformities in a 24-year-old white woman. A series of 12 cases of aortic medionecrosis and dissecting aneurysm recently reported from the Michael Reese Hospital[19] included two in which there was isthmic stenosis; the patients were 26 and 29 years of age. Of the 141 cases of dissecting hemorrhage in persons under 40 collected by Schnitker and Bayer,[2] 31.9% exhibited some degree of congenital aortic narrowing.

Coarctation of the aorta, mild in degree, was found post mortem in four cases of the present series; in two there was also aneurysmal dilatation of the proximal aorta preceding the fatal dissecting hemorrhage. Shennan[20] estimated in 1929 that more than 100 instances of rupture had been reported in cases of coarctation of the aorta; it cannot, therefore, be considered an unusual occurrence. Among 200 cases of coarctation of the aorta collected by Abbott,[21] spontaneous rupture had occurred in 38. It is unfortunate that microscopic examination was not made in each instance; but in 12 of the 13 cases with histologic data pronounced medial degenerative changes were found in areas adjacent to, but not involved in, the rupture. Pregnancy does not appear to influence the frequency of this complication,

14. Hall, E. M.: Healed Dissecting Aneurysm of the Aorta: Report of a Case with Patent Ductus Arteriosis and Enormous Hypertrophy of the Heart, Arch. Path. & Lab. Med. **2**:41-49, 1926.

15. Kinney, T. D.; Sylvester, R. E., and Levine, S. A.: Coarctation and Acute Dissection of the Aorta Associated with Pregnancy, Am. J. M. Sc. **210**:725-732, 1945.

16. Harrison, F. F.: Coarctation of the Aorta of the Adult Type Associated with Cystic Degeneration of the Media in the First Portion of the Arch, Arch. Path. **27**:742-747, 1939.

17. Yettra, M., and Lasky, I.: Aneurysmal Dilatation of Aorta Associated with Cystic Medial Necrosis: Case Report, Am. Heart J. **33**:516-522, 1947.

18. Milne, J. A.; Graham, J. G., and Simpson, J.: Cystic Mucoid Degeneration of the Aortic Media with Spontaneous Rupture of the Aorta, Glasgow M. J. **31**:206-211, 1950.

19. Amromin, G. D.; Schlichter, J. G., and Solway, A. J. L.: Medionecrosis of the Aorta, Arch. Path. **46**:380-385, 1948.

20. Shennan, T.: Dissecting Aneurysms, Medical Research Council, Special Report Series, No. 193, London, His Majesty's Stationery Office, 1934.

21. Abbott, M. E.: Coarctation of the Aorta of the Adult Type: A Statistical Study and Historical Retrospect of 200 Recorded Cases with Autopsy of Stenosis or Obliteration of the Descending Arch in Subjects Above the Age of 2 Years, Am. Heart J. **3**:574-618, 1928. Hamilton and Abbott.[13]

since rupture occurred in only 3 of 39 reported cases.[5] Blackford [22] related rupture of the aorta in coarctation to an imperfect development of the wall rather than to mechanical strain, because it occurred even when the stenosis was slight. In 24 of 104 autopsied cases of coarctation collected by Reifenstein, Levine, and Gross [23] subsequent to Abbott's [21] report, death was attributed to an abnormality of the vessel wall. The authors found that elastic-tissue destruction appeared to be the outstanding lesion, though they deplored the scarcity of detailed microscopic descriptions. It is noteworthy that hypertension was not found to be more frequent or severe in persons with rupture than in those without. As though to substantiate the secondary significance of hypertension, rupture had occurred distal to the coarctation in five cases. Halonen and Aho [24] reported a similar sequence in a 20-year-old white man with known coarctation; he died suddenly when an aneurysm distal to the obstruction ruptured.

Congenitally bicuspid aortic valves are commonly associated with coarctation. Abbott [21] found them to be present in 23.5% of her series, and among these cases, aortic rupture had occurred in 50%. Reifenstein, Levine, and Gross [23] found a similar association in 42.7% of their cases, an apparent increase related by Edwards and associates [4] to "the emphasis placed on the possible occurrence of this additional malformation by Abbott in 1928." This deformity was present in nine cases of this series but was associated with coarctation only once. In four persons with this anomaly, aneurysmal dilatation of the ascending portion of the aortic arch had preceded the terminal rupture or dissection (Figs. 1, 2, and 3).

Arachnodactyly, a developmental deformity of the hands and feet, known as Marfan's syndrome when associated with dislocation of the lens, was present in three of this series. Baer, Taussig, and Oppenheimer [25] attributed the aortic aneurysms in two instances of this condition to a congenital malformation of the media. The literature regarding the Marfan syndrome has been critically reviewed by Tobin, Bay, and Humphreys.[5] Two patients on whom they reported, a man of 22 and a woman of 35, both white, died with dissecting aneurysms which arose in each instance from a considerably dilated ascending aorta. Fibromyxomatous thickening of the cardiac valves in the second case was interpreted by the authors as a congenital defect; the cystic medial degeneration seen in their illustrations coincides with the type classified here as primarily elastic. Postmortem examination in 14 cases revealed degenerative aortic lesions in 6. The ages of the patients with such lesions ranged from 14 to 35 years, whereas five of the remaining eight patients were less than 28 months of age. It could reasonably be inferred that increasing age provides further opportunity for the morphologic manifestations of the underlying defect.

22. Blackford, L. M.: Coarctation of the Aorta, Arch. Int. Med. **41**:702-735, 1928.

23. Reifenstein, G. H.; Levine, S. A., and Gross, R. E.: Coarctation of the Aorta: A Review of 104 Autopsied Cases of the "Adult Type" 2 Years of Age or Older, Am. Heart J. **33**:146-168, 1947.

24. Halonen, P. I., and Aho, A.: Coarctation of the Thoracic Aorta with an Aneurysm Distal to the Obstruction, Acta path. et microbiol. scandinav. **26**:77-82, 1949.

25. Baer, R. W.; Taussig, H. B., and Oppenheimer, E. H.: Congenital Aneurysmal Dilatation of the Aorta Associated with Arachnodactyly, Bull. Johns Hopkins Hosp. **72**:309-331, 1943.

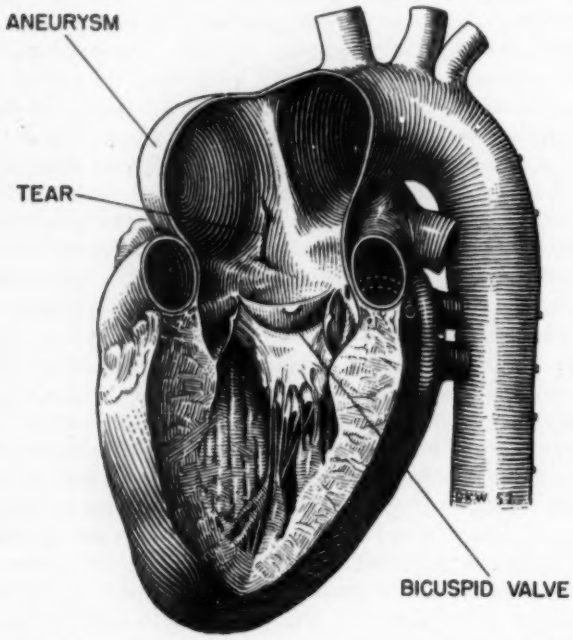

Fig. 2 (Case 10).—Anterolateral view of the left ventricle and aorta. The pulmonary artery has been transected and retracted to expose the outflow tract. Observe the aneurysmal dilatation of the proximal aorta, the longitudinal tear on its posterior wall, and the bicuspid aortic valve. Death resulted from intrapericardial hemorrhage.

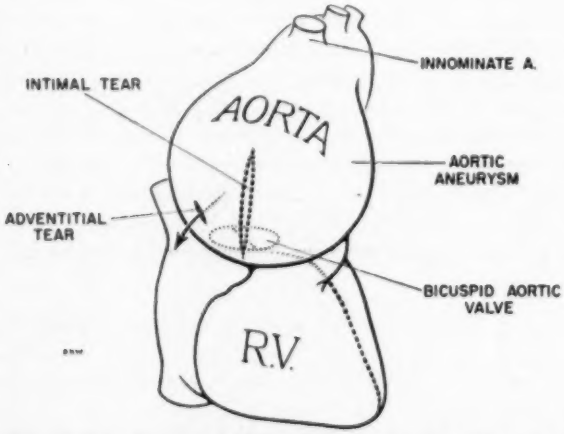

Fig. 3 (Case 5).—As in Figure 2, the features to be observed are the large aneurysm of the proximal aorta, a longitudinally directed intimal tear on its posterolateral wall, the bicuspid aortic valve, and the adventitial tear, which contributed to the fatal intrapericardial hemorrhage.

Recognition of the association of congenital anomalies with deficiencies of the vessel wall has practical significance to the physician concerned with the functional, if not the anatomic, correction of the deformity. Moreover, though the present discussion is concerned primarily with the aorta, the vascular defects are not similarly limited. Wilkinson [26] noted the presence of a bicuspid pulmonic valve in a 13-year-old girl who had died promptly after spontaneous rupture of an aneurysm of the left pulmonary artery. In 1939, Boyd and McGavack [27] found that congenital anomalies were present in 66% of 111 cases of aneurysm of the pulmonary artery reported since 1833. Mackler and Graham [28] observed that aneurysms of Botallo's duct may occur in adults (5 cases) as well as in infants (24 cases). Congenital aneurysms of the cerebral arteries are not uncommon; fatal intracranial hemorrhages, usually subarachnoid, occurred in 7% of patients with coarctation.[4] Gardner and Hardwick [29] found an aneurysm of the left middle cerebral artery in a man 67 years of age who had died when a dissecting aortic aneurysm of five days' duration ruptured into the pleura. They considered it possible that the origin of both lesions had a common basis. Boyd and Werblow [30] reported a somewhat similar occurrence in a white housewife of 57 years who died from intrapericardial hemorrhage. Postmortem examination disclosed a dissecting aneurysm of a dilated ascending aorta, severe isthmic stenosis, and aneurysmal dilatation of the left vertebral artery. In one of the cases of nonsyphilitic aneurysm reported by Steinberg, Grisham, and Sussman,[31] a 44-year-old man with an aneurysm at the bifurcation of the right innominate artery had a history of excision of a tibial aneurysm 12 years earlier and of a popliteal aneurysm 4 years earlier.

The seven instances of true aneurysmal dilatation of the ascending aorta in the present series provide some interesting information regarding the rapidity with which the underlying medial degeneration developed. All of them occurred in persons who had served in the United Statees Army during World War II. At the time of death each had an aortic lesion of such size that a considerable deformity would probably have been manifest in a roentgenogram of the chest. Such a deformity apparent at the time of the induction examination would have precluded acceptance into the military service; therefore it is reasonable to conclude that the aneurysm appeared during the period of service, or at least that it had been overlooked because of its small size and had grown rapidly before the patient's death. In two of the cases it was possible to secure roentgenograms which demonstrated the veracity of this assumption. In one, that of a white man 23 years of age (Case 5), the roentgenogram of the chest was essentially normal at the time of induction;

26. Wilkinson, K. D.: Aneurysmal Dilatation of the Pulmonary Artery, Brit. Heart J. **2**:255-259, 1940.

27. Boyd, L. J., and McGavack, T. H.: Aneurysm of Pulmonary Artery: Review of the Literature and Report of 2 New Cases, Am. Heart J. **18**:562-578, 1939.

28. Mackler, S., and Graham, E. A.: Aneurysms of the Ductus Botalli as a Surgical Problem, J. Thoracic Surg. **12**:719-727, 1943.

29. Gardner, E., and Hardwick, S. W.: Dissecting Aneurysm of the Aorta Associated with Intracranial Aneurysm and Cerebral Hemorrhage, J. Path. & Bact. **58**:289-293, 1946.

30. Boyd, L. J., and Werblow, S. C.: Coarctation of the Aorta, Dissecting Aneurysm, and Aneurysmal Dilatation of the Left Vertebral Artery: Report of a Case, Ann. Int. Med. **11**: 845-850, 1937.

31. Steinberg, M. F.; Grisham, A., and Sussman, M. L.: Non-Syphilitic Aneurysm of the Aorta in Individuals Under 45 Years of Age, J. Thoracic Surg. **12**:704-713, 1943.

nine months later postmortem examination disclosed an aneurysm of the ascending aorta which approximated in size the heart itself. In the second case, that of a white man 25 years of age (Case 9), no significant aortic thickening was observed at induction, but 32 months later, when he was separated from the army, an aneurysm of considerable size was detected. This patient died when a huge aneurysm of the ascending aorta ruptured 22 months after he had left military service. Magner and McKenzie [32] have described similar growth of an aneurysm of the ascending aorta over a period of three and one-half years in a 20-year-old white man. Inasmuch as syphilis is the most frequent cause of aneurysm of this portion of the aorta, it seems relevant to mention that neither the clinical history nor the pathologic findings suggested such an origin in any of the cases briefly outlined in Table 3.

COMMENT

The frequency with which congenital anomalies are to be observed with dissecting aortic aneurysms in the younger age groups, and the high proportion of persons with developmental deformities who also have deficiencies of the aortic wall, suggest a common, or at least a closely related, etiologic background. However, the evidence also suggests that the morphologic defect in the media may not become manifest until some time after birth. As was mentioned previously, pathologic studies of the Marfan syndrome showed defects of the aortic wall to be more common among the older persons affected. The present study includes several cases in which there was good reason to believe that the weakening of the aortic wall leading to the formation of aneurysm had appeared or had become greatly accentuated relatively late in postnatal life.

Morphologic normality in utero and at birth is not necessarily inconsistent with the congenital origin of a defect which appears subsequently; witness the months or years that may elapse before arachnodactyly may be recognized. The lesions in pseudoxanthoma elasticum and its associated congenital affliction, angioid streaks of the retina, may not become manifest until late adult life, though they are usually present by puberty.[33] Similarly, Schut,[34] in tracing a form of hereditary ataxia through six generations of an afflicted family, observed that for a number of years after birth normality was the rule. The primary lesion under such circumstances must be of a subtle biochemical or metabolic character, since years may elapse before structural changes appear. Such a hypothesis would seem tenable in the cases here discussed.

The nature of this lesion is, of course, even more speculative. In reference to the type of degeneration conspicuous in dissecting aneurysms occurring under the age of 40, a slightly defective or inferior metabolism for the maintenance of elastic-tissue integrity can be postulated. As a result there is premature aging, or perhaps an increased vulnerability to metabolic, chemical, or nutritional interference. This observation recalls immediately that loss of elasticity is a part of "normal" senescence.

32. Magner, D., and McKenzie, H. F.: Cystic Medionecrosis of the Aorta, Arch. Path. **44**: 485-489, 1947.

33. Benedict, W. L., and Montgomery, H.: Pseudoxanthoma Elasticum and Angioid Streaks, Am. J. Ophth. **18**:205-212, 1935.

34. Schut, J. W.: Hereditary Ataxia: Clinical Study Through 6 Generations, Arch. Neurol. & Psychiat. **63**:535-568, 1950.

Holman [35] produced arterial lesions through a combination of dietary measures and renal insufficiency (though the experimental lesions were diffuse and acutely inflammatory). It is significant, too, that pyridoxine deficiency in monkeys results in the accumulation of basophilic "ground substance" in the vessel walls.[36] The special vulnerability of the proximal aorta may be explained as a reflection of the more intense physiologic activity and the consequently greater metabolic requirements of this segment. In the case of Balzar, cited by Benedict and Montgomery,[33] the skin lesions of pseudoxanthoma elasticum were accompanied by elastic-tissue defects in the heart, indicating that the process may be widespread. Wallach and Burkhart [37] have recently reported an example of the Ehlers-Danlos syndrome associated with the tetralogy of Fallot. Clearly, morphologic studies are required to indicate the lines of biochemical research essential to an understanding of these lesions. Merely to classify them as congenital mesodermal inadequacy, as Holle [9] has done, though not inaccurate, would not fulfill the proper function of morphologic investigation. The concept suggested in 1939 by Hass,[38] that elastic tissue maintains a metabolic relationship with the components of the medium in which it lies, has not always been fully appreciated. The relative resistance exhibited by elastic fibers to necrosis or digestion has misled pathologists generally to regard the tissue as essentially inert.

SUMMARY

A group of 32 cases of dissecting aortic aneurysms in persons younger than 40 years has been reviewed. Symptomatically the process was indistinguishable from that in an older age group, but histologically there was a distinct predominance of the type of medial degeneration affecting the elastic laminae. An unusually large number of congenital abnormalities were associated with the vascular lesions. In two cases roentgenologic evidence of the development of an aneurysm over a period of months was reported, and the same sequence could be inferred in five other cases. In view of these observations, it seems unreasonable to assume that a morphologic defect was present at birth. It is more likely that the congenital lesion was a metabolic one which required time and unknown factors for its exploitation and the development of secondary morphologic manifestations.

35. Holman, R. L.: Acute Necrotizing Arteritis, Aortitis, and Auriculitis Following Uranium Nitrate Injury in Dogs with Altered Plasma Proteins, Am. J. Path. **17**:359-376, 1941. Holman, R. L.: Necrotizing Arteritis in Dogs Related to Diet and Renal Insufficiency: Evidence for a Dietary Factor, Am. J. Path. **19**:977-991, 1943. Holman, R. L., and Swanton, M. C.: "Dietary Factor" in Necrotizing Arteritis in Dogs a Lipid Substance, Proc. Soc. Exper. Biol. & Med. **63**:87-89, 1946.

36. Rinehart, J. F.: Vascular Lesions in Pyridoxine Deficient Monkeys, read before the American Association of Pathologists and Bacteriologists, Boston, April, 1949.

37. Wallach, E. A., and Burkhart, E. F.: Ehlers-Danlos Syndrome Associated with the Tetralogy of Fallot, Arch. Dermat. & Syph. **61**:750-752, 1950.

38. Hass, G. M.: Elastic Tissue, Arch. Path. **27**:334-365; 583-613, 1939.

PATHOLOGY OF A PNEUMOTROPIC VIRUS RECOVERED FROM C3H MICE CARRYING THE BITTNER MILK AGENT

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BETHESDA, MD.

IN A PREVIOUS communication [1] a virus is described which is apparently latent in adult C3H mice carrying the Bittner milk factor. This agent causes a fatal pneumonitis associated with viremia on inoculation into young suckling mice. Isolations of this agent, provisionally referred to as the K virus, have been made from pooled liver and spleen and mammary tissue of adult C3H mice on three separate occasions. In all instances, the virus was recovered on first passage in suckling mice. Once established, the virus is uniformly fatal for suckling mice of different strains in approximately eight or nine days. Repeated attempts to infect older (over 10-day-old) suckling or adult mice or to infect guinea pigs, hamsters, or embryonated eggs have been uniformly unsuccessful. It is unknown at present whether the K virus is related to the Bittner milk factor. The present paper describes pathologic changes in the lung produced by K virus in suckling mice. These lesions are quite distinct from other types of mouse pneumonitis which we have had the opportunity to study.

MATERIAL AND METHOD

Suckling and adult mice of C3H and regular Swiss strains were utilized in this study. Fifteen received K virus intracerebrally, five intraperitoneally, ten by both the intraperitoneal and the intracerebral route, five intranasally, and five orally. Tissues were studied 4 to 13 days after inoculation. Sections of brain, lungs, liver, heart, and on occasion kidney, adrenal, gastrointestinal tract, and spleen were fixed in either 10% formalin or Zenker's solution, in the former for 16 hours and in the latter for 4 hours, and then washed for 16 hours in running water. Sections were stained routinely with hematoxylin and eosin and by the Wolbach-Giemsa method. Many sections were stained by the Feulgen nucleal procedure, periodic acid-Schiff method, Brown-Brenn bacterial stain, and Ziehl-Neelsen acid-fast method. Frozen as well as paraffin sections were stained by the Oil Red O and Sudan black B methods for lipids. Sections were also, on occasion, treated with crystalline ribonuclease 1:10,000 in pH 6 phosphate buffer for one hour at 37 C.

Sections of tissues from uninoculated suckling mice of comparable age to those inoculated were examined as controls. In addition, tissues of adult female Bittner mice were studied, of which three were pregnant and five lactating. As further controls, four suckling mice were inoculated intranasally with the Nigg pneumonitis virus.

From the Laboratory of Pathology and Pharmacology, National Institute of Arthritis and Metabolic Diseases, and the Laboratory of Infectious Diseases, National Microbiological Institute, National Institutes of Health.

1. Kilham, L.: Isolation in Suckling Mice of a Virus from C3H Mice Harboring Bittner Milk Agent, Science, to be published.

RESULTS

Morphologic changes were similar after intracerebral, intraperitoneal, intranasal, or oral administration of K virus. The outstanding histologic change in infected lungs was a "ballooning" of the nuclei of endothelial cells, most marked in vessels of small caliber, although not absent in the larger muscular vessels (Figs. 1 and 2). Such cells were two to four times as large as those observed in control sections and were round to ovoid in shape. Their nuclei were large and vacuolated, with fragments of chromatin deposited about the nuclear membrane. This nuclear "ballooning" was not as pronounced in formalin-fixed tissue as it was in sections prepared

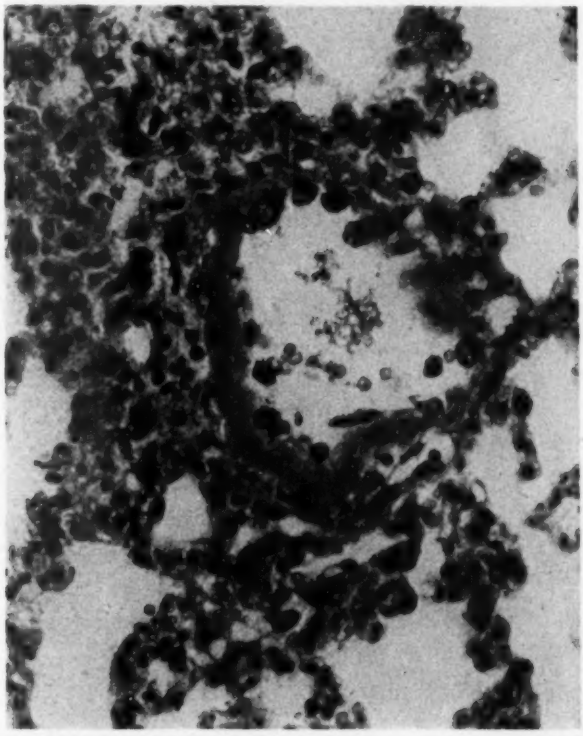

Fig. 1.—Endothelial cells of large vessels are swollen, and their nuclei appear vacuolated. Septal infiltrate is mononuclear. × 385.

after Zenker's fixation. Cell cytoplasms were basophilic and scanty. Within the clear portion of the nucleus a round and occasionally ovoid or polygonal homogeneous body measuring 4 to 8 μ in diameter was frequently observed (Fig. 3). Rarely, several were present within the same nucleus. They appeared plum-colored after hematoxylin and eosin and blue when stained by the Wolbach-Giemsa method. These bodies demonstrated a positive Feulgen nucleal reaction (Fig. 4). They were unaffected by ribonuclease and failed to stain by the Brown-Brenn method for bacteria, the Ziehl-Neelsen acid-fast method, the Oil Red O and Sudan black B methods for lipids, and the periodic acid-Schiff procedure.

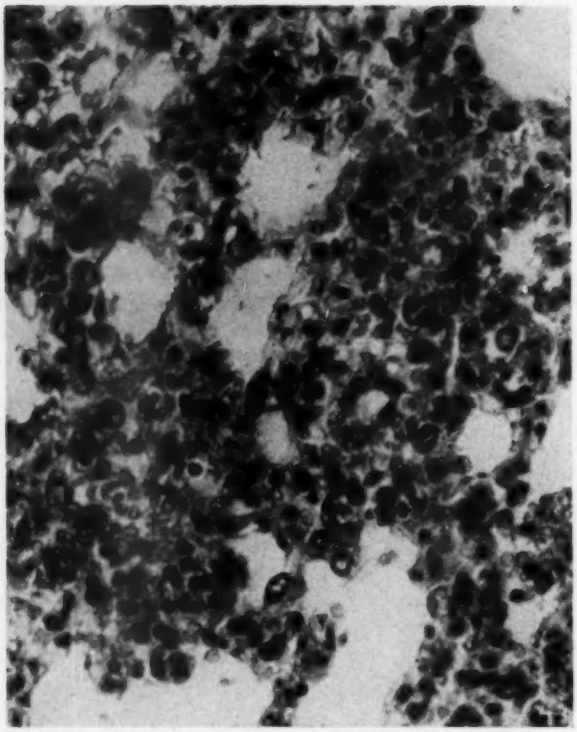

Fig. 2.—Septal capillaries demonstrate large endothelial cells. × 965.

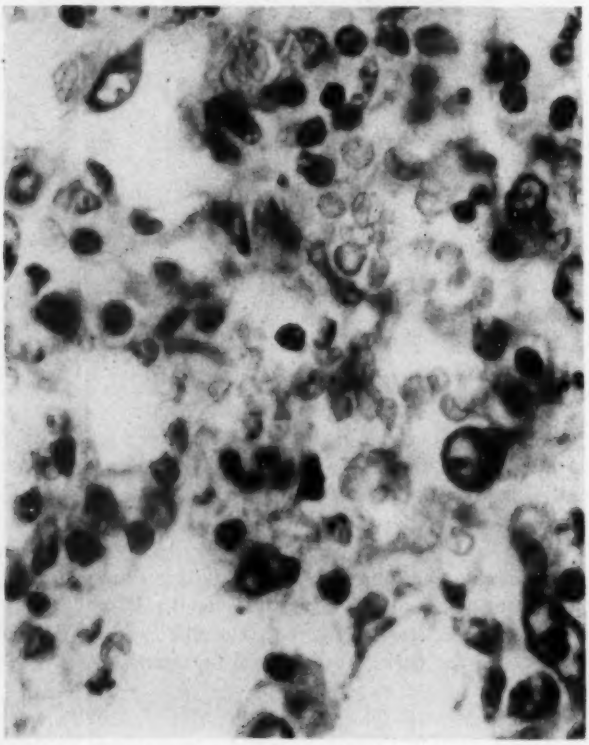

Fig. 3.—Higher magnification, revealing intranuclear inclusions especially marked at the lower right. × 1,480.

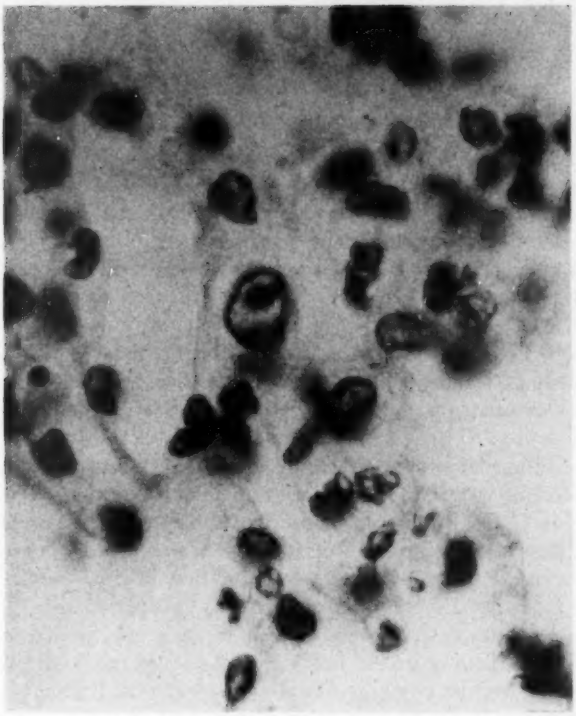

Fig. 4.—Section stained by the Feulgen nucleal procedure, demonstrating a positive intranuclear body. The cell appears to lie free in a septal space. × 1,120.

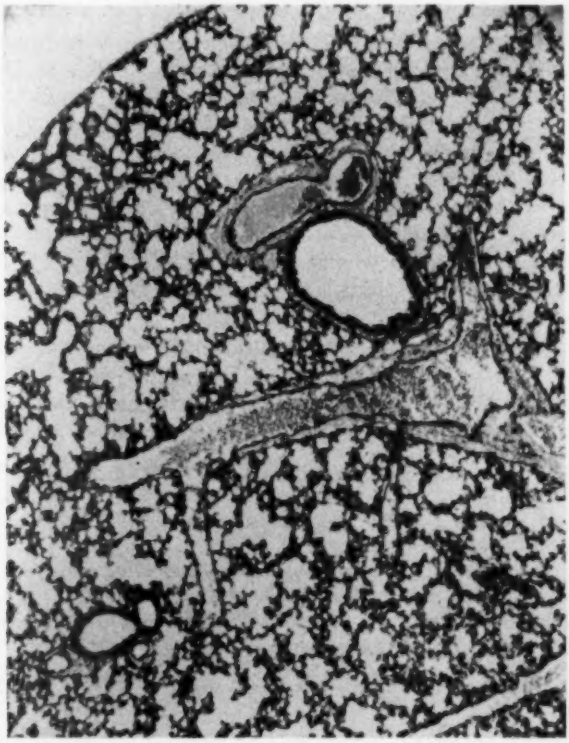

Fig. 5.—Low-power photomicrograph demonstrating uninvolved pleura and bronchi. The alveoli appear free. Actual pathologic change is not distinct at this magnification, although the septa are somewhat thickened. × 65.

Septal vessels of the lungs in many instances were occult owing to occlusion of the lumina by these large endothelial cells. In addition, some of these cells appeared to assume positions along the alveolar lining. The septa also contained a moderate lymphocytic and histiocytic infiltrate. A rare neutrophile was present in the septal wall. There was no fibroblastic proliferation observed within the septa. The alveolar spaces were for the most part empty except for an occasional acidophilic coagulum, but inflammatory cells were lacking. All pulmonary changes were diffuse. The pleura and bronchi were free of abnormality (Fig. 5).

After intraperitoneal passage, an occasional sinusoidal cell of the liver revealed nuclear "ballooning" and contained a nuclear body similar to those described in the lung. Sections of brain, heart, kidney, spleen, and adrenal failed to reveal morphologic abnormality.

Lesions could not be produced in adult mice or in suckling mice older than 10 days, and no lesions were evident in susceptible animals killed earlier than 5 days after inoculation. There was no definite correlation between the developmental phase and extent of the pulmonary changes produced and the time interval after inoculation. The intranuclear bodies were apparent at all time intervals after five days and demonstrated a positive Feulgen nucleal reaction at all periods.

Examination of tissues from uninoculated suckling mice and control lactating as well as pregnant Bittner mice failed to reveal any morphologic changes. Neoplastic and normal mammary tissue likewise failed to reveal cellular inclusions or endothelial change.

COMMENT

Pulmonary changes produced by K virus in mice are unlike those described with other pneumonitis viruses, such as PVM, influenza, and the meningopneumonitis-psittacosis group.[2] Nigg pneumonitis virus produces alveolar consolidation as well as septal infiltration with lymphocytes and monocytes and frequently polymorphonuclear leucocytic infiltration.[3] On occasion there is perivascular cuffing and hyaline-membrane formation in the alveoli. Pulmonary changes are frequently patchy, and areas may reveal various stages in the inflammatory process. A similar spotty pneumonic process is also pronounced in psittacosis pneumonitis. In addition, de Gara and Furth [4] have described the presence of elementary bodies in the meningopneumonitis group. These were present in both monocytes and neutrophiles early in the disease process. Such bodies have been described in lesions caused by psittacosis and the pneumonitis virus of Nigg.

In none of the foregoing, however, is endothelial swelling a distinct feature of the pathologic picture; yet such change is outstanding in lesions produced by K virus. Although swollen endothelial cells appear to line occasional alveoli, and a slight acidophilic coagulum is sometimes present, the alveoli are generally clear.

2. Magill, T. P.: The Pathogenesis and Pathology of Viral Diseases, New York Academy of Medicine Symposium, No. 3, Columbia University Press, 1950, p. 152. Horsfall, F., and Hahn, R.: Latent Virus in Normal Mice Capable of Producing Pneumonia in Its Natural Host, J. Exper. Med. **71**:391, 1940.

3. Nigg, C., and Eaton, M.: Isolation from Normal Mice of a Pneumotropic Virus Which Forms Elementary Bodies, J. Exper. Med. **79**:497, 1944.

4. de Gara, P. F., and Furth, J.: Pneumonia Produced by Meningopneumotropic Virus; Report of Fatal Case, with Observations on Inter-Relationship of Psittacosis-like Viruses, Arch. Path. **45**:474, 1948.

There is no perivascular infiltrate, and inflammatory changes are limited to the septa. Polymorphonuclear leucocytes are rare. The endothelial swelling is found in all lung fields and is uniform in appearance. Also, the presence of intranuclear inclusions has not been described in the psittacosis-like group. These bodies demonstrated a positive Feulgen nucleal reaction, revealing their desoxyribosenucleic acid content. The significance of intranuclear bodies has long been a source of debate; however, considering the morphologic picture as well as the histochemical definition, one may assume that such structure represents an alteration in nuclear substance. Although most cellular inclusions are considered to be Feulgen-negative, Crouse and associates [5] have demonstrated desoxyribosenucleic acid in the intranuclear inclusions of herpes simplex. Elementary bodies were not observed in the lesions produced by the K virus.

The behavior of the K virus is quite distinctive. To our knowledge no pneumotropic virus of murine origin has been described which will lead to illness and death in suckling but not in adult mice. What role, if any, this virus plays in tumor genesis is now being studied.

SUMMARY

A virus which is apparently latent in C3H mice carrying the Bittner milk factor has been found to produce fatal pulmonary lesions when inoculated by a variety of routes into suckling C3H or Swiss mice.

The pulmonary changes are unlike those described for other pneumotropic viruses in mice. They consist principally of marked swelling of the endothelial cells of large and small vessels accompanied by a moderate lymphocytic and monocytic septal infiltrate. The nuclei of the endothelial cells appear vacuolated and in many instances contain round, ovoid, or polygonal bodies which stain positively with the Feulgen nucleal procedure and may therefore represent altered nuclear substance.

No evidence of disease or similar endothelial changes has been noted in control sections obtained from uninoculated suckling mice of C3H or Swiss strains or from tissues of adult lactating or pregnant Bittner mice. Study of mammary tumors from a few of these animals also failed to reveal any histologic evidence of virus disease.

5. Crouse, H. V.; Coriell, L. L.; Blank, H., and Scott, T. F.: Cytochemical Studies on the Intranuclear Inclusion of Herpes Simplex, J. Nat. Cancer Inst. **10**:1372, 1950.

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HISTOCHEMICAL STUDIES ON THE LENS FOLLOWING RADIATION INJURY

SOLBERT PERMUTT, M.D.
AND
FRANK B. JOHNSON, M.D.
CHICAGO

MANY COMPLEX organic compounds have been shown to fragment or split when exposed to various types of irradiation in vitro.[1] Therefore, it was felt that some of the in vivo effects of irradiation might ultimately be explained in terms of depolymerization of certain constituents of tissues. Our studies have been carried out with special emphasis on the polysaccharide-protein components of the lens. In many respects the lens is an ideal organ for such investigation. Krause[2] stated: "The lens is a unique tissue which lends itself to chemical study as no other tissue can. It is biologically like an avascular tissue culture which furnishes a pure isolated colony of cells." High levels of radiation may be administered to the lens with minimal exposure of other organs, thereby avoiding the severe systemic effects of irradiation.

This report will attempt to (1) characterize the polysaccharide-protein components of the normal lens by histochemical procedures, (2) demonstrate alterations of these components following x-ray and slow-neutron irradiation, and (3) discuss possible relations between these alterations and the pathogenesis of radiation cataracts.

GENERAL METHODS

The normal lens was studied in adult male rabbits weighing 2 to 5 kg., Sprague-Dawley rats of approximately 150 gm., and white mice of approximately 20 gm. The effects of x-rays and slow-neutron radiation were studied only in rabbits. Two rabbits, each with one eye exposed to x-rays, were obtained from Dr. David G. Cogan, of the Howe Laboratory of Ophthalmology, Harvard University Medical School. Rabbit H-1 was 3 years old at the time of irradiation and received 1,500 r of 1.2 mev. energy to the right eye 230 days before study. Rabbit H-2 was 10 weeks old at the time of irradiation and received 1,500 r of 1.2 mev. energy to the right eye 160 days before study. Six 10-week-old rabbits whose eyes were exposed to slow neutrons

From the Department of Anatomy, University of Chicago.

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1. Baker, L. E., and Corey, R. S.: J. Exper. Med. **50**:439, 1929. Zhukov, I. I., and Unkovskaya, V. A.: J. Russ. Phys. Chem. Soc. **62**:581, 1930; Chem. Abst. **24**:4708, 1930. Loiseleur, J.: Compt rend. Acad. sc. **201**:1511, 1935. Mitchell, J. S.: Proc. Roy. Soc., London, s. A. **155**:606, 1936. Carpenter, D. C.: Science **89**:251, 1939. Svedberg, T., and Brohult, S.: Nature, London **143**:938, 1939. Sanigar, E. B.; Krejci, L. E., and Kraemer, E. O.: Biochem. J. **33**:1, 1939. Khenokh, M. A.: J. Gen. Chemistry, Moscow **11**:776, 1941; C. A. **36**:2473, 1941. Davis, B. D.; Hollaender, A., and Greenstein, J. P.: J. Biol. Chem. **146**:663, 1942.

2. Krause, A. C.: Biochemistry of the Eye, Baltimore, Johns Hopkins Press, 1934, p. 186.

were obtained from Dr. John L. Goff, Los Alamos Medical Center, Los Alamos, N. M. These animals were killed approximately 250 days after receiving slow-neutron fluxes varying from 2.08×10^{10} n/cm.^{2} to 2.41×10^{11} n/cm.^{2}

The lenses were studied ophthalmoscopically. The animals were then killed by a sharp blow and were bled. The lenses were removed as rapidly as possible and immediately frozen by immersion in isopentane at about —155 C. They were dried in a vacuum at about —30 C., infiltrated in vacuo with paraffin (melting point 56 to 58 C.) for 15 minutes, and embedded. Sections of small pieces, 6 to 10 μ, were cut and mounted on slides with finger pressure.

Sections were stained for polysaccharide by the periodic acid-leucofuchsin method as suggested by Hotchkiss.[3] According to Gersh,[4] this method may be considered fairly specific for polysaccharide-protein complexes if glycogen is removed by the use of saliva after protein coagulation and if lipids are removed by extraction with hot methanol-chloroform for 16 hours. The possible role of some proteins has to be considered because of the reactivity with periodic acid-leucofuchsin of several amino acids, e. g. serine, threonine, and hydroxylysine. Hotchkiss[3] has pointed out, however, that "that hydroxyamino acid residues of proteins (probably excepting hydroxylysine) are chemically substituted so that free glycol is not present, and do not give the reaction." Hydroxylysine is generally considered to be rare in animal proteins.[5]

One of the difficulties in the interpretation of positive or negative sites of stainability is the uncertainty of knowing how much of the reactive material was dissolved out by the reagents employed in the Hotchkiss procedure. It was found that lyophilyzed lens is nearly completely dissolved in distilled water after being stirred in a Waring blendor.® If the solution is acidified, precipitation occurs, the maximum being at pH 3.5. If the pH is reduced below 3.5, the lens material is again soluble. Most of the reagents of the standard Hotchkiss procedure have a pH considerably lower than 3.5. It was found that if the reagents used are adjusted to a pH of 3.5, more intense staining of the lens components occurs, and, in addition, some structures stain which are unstained by the more acidic reagents. It appears, therefore, that considerable amounts of polysaccharide-protein complexes of the lens are soluble in the reagents when prepared according to the method of Hotchkiss.

We modified the Hotchkiss procedure in order to keep at a minimum the loss of reactive components from the lens due to solubility in the reagents. The details of our procedure are as follows: (1) Periodic acid, leucofuchsin, and sulfite rinse are prepared according to the directions of Hotchkiss (the alcoholic periodic acid being used), except that 1 M sodium acetate is added to each reagent immediately before using until the pH is adjusted to 3.5; (2) the sections are placed in the periodic acid for 5 minutes, (3) into 10 changes of 70% alcohol for a total of 5 minutes (instead of a reducing rinse), (4) into leucofuchsin for 15 minutes, (5) into 3 changes of sulfite rinse for 5 minutes each, and then (6) the sections are run up through xylene and mounted in "clarite." Attention is called to the fact that the leucofuchsin when adjusted to a pH of 3.5 is pink. We have found that this does not interfere with the specificity of the stain. According to Arzac,[6] even the direct use of colored basic fuchsin gives results identical with those obtained with leucofuchsin.

Sections deparaffinized in several changes of petroleum ether and dried by gently warming were stained for metachromasia with 0.05% aqueous toluidine blue. The stain was applied directly to the deparaffinized sections, and after five minutes the excess was removed, a coverslip applied, and the sections examined immediately for metachromasia.

In order to ascertain the solubility of the polysaccharide-protein complexes under varying conditions, sections were treated with various solutions and enzyme preparations before or after alcohol coagulation and before staining by the above methods.

Ultraviolet absorption of lens fibers on sections 2 μ thick was measured at 2,804 A. as a means of determining changes in protein concentration, according to the method of Catchpole and Gersh. [7]

3. Hotchkiss, R. D.: Arch. Biochem. **16**:131, 1948.

4. Gersh, I.: Arch. Path. **47**:99, 1949.

5. Leblond, C. P.: Am. J. Anat. **86**:1, 1950. Van Slyke, D. D.; Hiller, A., and MacFadyen, D. A.: J. Biol. Chem. **141**:681, 1941.

6. Arzac, J. P.: Stain Technol. **25**:187, 1950.

7. Catchpole, H. R., and Gersh, I.: Discussions of the Faraday Society, No. 9, 1950, p. 481.

RESULTS IN NORMAL LENS

Histochemical Localization of Polysaccharide-Protein Complexes.—With the modified Hotchkiss technique, staining occurred in four parts of the normal lens (Fig. 1). The capsule of the lens showed an intense and uniform reaction. The cytoplasm of the subcapsular epithelial cells contained granules with a moderately intense red. The cytoplasm of the lenticular fibers showed a faint and uniform pink. The cement substance of the lens fibers was red. Nuclei were unstained. The reacting material was probably polysaccharide-protein in nature. The lipids had been extracted for 16 hours with hot methanol-chloroform. Glycogen has not been found on chemical examination.[8]

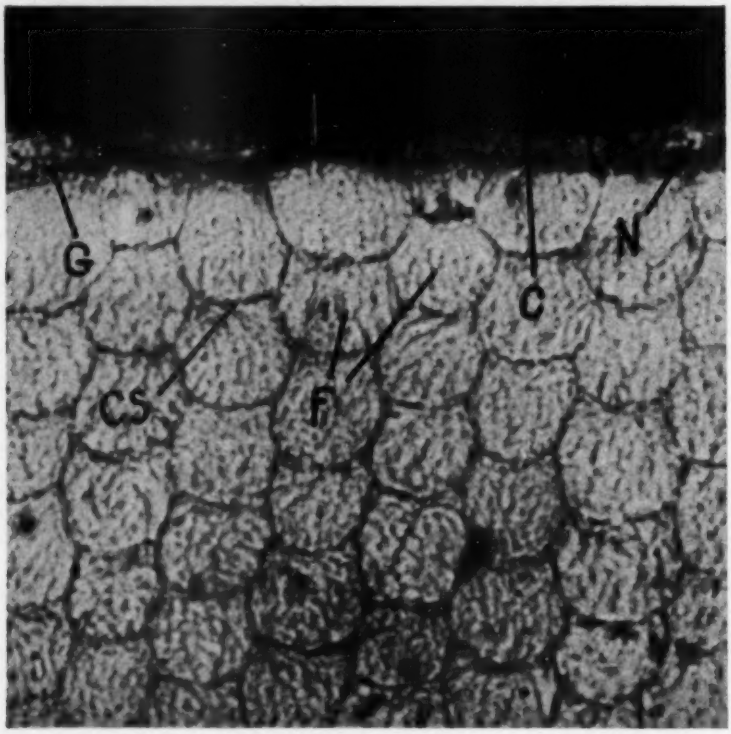

Fig. 1.—Photomicrograph of normal lens of the rabbit. The specimen was fixed by freezing and drying, sectioned, and stained by the periodic acid-leucofuchsin method after extraction with methanol-chloroform for 16 hours. The lenticular fibers were cut transversely. *C* indicates the capsule; *N*, a nucleus of the subcapsular epithelium; *G*, granules in the epithelial cells; *F*, one of the transversely sectioned fibers, and *CS*, cement substance. × 1,500.

Staining with aqueous toluidine blue resulted in metachromasia of the capsule, the cement substance, and the nuclei. Metachromatic granules were found in the cytoplasm of the epithelium. The lenticular fibers were, however, orthochromatic.

8. Leber, A. T.: von Graefes Arch. Ophth. **62**:85, 1906.

Solubility of the Polysaccharide-Protein Complexes.—If the periodic acid-leucofuchsin method was used with frozen-dried lens according to the directions of Hotchkiss, only the capsule stained. Leblond,[5] using the regular Hotchkiss technique on material fixed in Orth's fluid, also made this observation. This fact would seem to indicate that extraction of considerable quantities of polysaccharide-protein complexes occurs during the regular Hotchkiss procedure or during fixation. A more detailed study of the extractability of the polysaccharide-protein complexes was made in an attempt to characterize them.

Sections were immersed in carbon-dioxide-free distilled water at pH 7.0 or in 0.01 M phosphate buffer at pH 7.0 to determine water solubility. Other sections were placed in hydrochloric acid at pH 1.0 to determine acid solubility. The sections were left in one of the aforementioned solutions for one hour either before or after alcohol coagulation and then stained by the modified Hotchkiss procedure. The results are set forth in Table 1 *A*.

TABLE 1.—*Solubility of Various Hotchkiss-Positive Components of the Lens in Water, Phosphate Buffer at pH 7.0, and Hydrochloric Acid at pH 1.0, for One Hour**

		CO_2-Free H_2O pH 7.0	1/100 M Phosph. Buffer pH 7.0	HCl pH 1.0
A.	Normal Lens			
	Capsule	+	+	+
	Cement substance	+	+	+
	Granules in epithelial cells	−	−	−
	Lenticular fibers	−	−	−
B.	Cataractous Lens			
	Capsule			
	Exterior portion	+	+	+
	Interior portion	−	−	−
	Cement substance	−	−	−
	Lenticular fibers	−	−	−

* A plus sign indicates that staining was approximately the same as in the unextracted control; a minus sign, that staining was markedly decreased or absent as compared with that in the unextracted control.

From these results, the polysaccharide-protein complexes of the lens can be divided into two classes on the basis of solubility: 1. The polysaccharide-protein material in the capsule and cement substance is insoluble in water at pH 7.0 and hydrochloric acid at pH 1.0 in sections of frozen-dried lens. 2. The polysaccharide-protein material in the epithelial cells and the lenticular fibers is soluble in water at pH 7.0 and hydrochloric acid at pH 1.0 in sections of frozen-dried lens. (Solubility was greatly reduced at pH 3.5, thereby allowing staining with the modified Hotchkiss procedure.)

The cement substance, which was insoluble in water at pH 7.0 and hydrochloric acid at pH 1.0, was soluble in the reagents of the regular Hotchkiss procedure. This fact was apparently due to a modification of the cement substance after periodic acid oxidation to such a state that it became soluble in the acidic reagents of the regular Hotchkiss procedure but remained insoluble at pH 3.5.

Enzyme Studies on the Polysaccharide-Protein Complexes.—The following enzymes were allowed to act on sections by the hanging-drop method, both prior to and after alcohol coagulation at 37 C.: hyaluronidase (partially purified and lyo-

philized),[9] collagenase,[10] lysozyme purified from egg white,[11] pepsin (purified),[11] and trypsin (purified).[11] After this procedure the sections were stained by the modified Hotchkiss procedure.

The subcapsular epithelial cells and fibers were not stained in the control sections, as the reacting substances in these areas were soluble (Table 1). The results of the various enzymes on the polysaccharide-protein complexes of the capsule and cement substance in sections of frozen-dried specimens are set forth in Table 2. Hyaluronidase and lysozyme had no effect on the stainable components of the capsule and cement substance. Collagenase reduced only slightly the amount of reactive material of the capsule; it reduced markedly the amount of reactive material of the cement substance. Pepsin and trypsin removed completely the reactive material of both the capsule and the cement substance.

TABLE 2.—*Effects of Various Enzyme Preparations on the Hotchkiss-Positive Components of the Normal Lens**

Enzyme	Capsule	Cement Substance
Hyaluronidase: 1 mg./cc. in phosphate buffer, pH 7.0, for 1 hr.	+	+
Control: phosphate buffer, pH 7.0, for 1 hr.	+	+
Collagenase: 1 mg./cc. in phosphate buffer, pH 7.0, for 1 hr.	±	−
Control: phosphate buffer, pH 7.0, for 1 hr.	+	+
Lysozyme: 1 mg./cc. in phosphate buffer, pH 7.0, for 1 hr.	+	+
Control: phosphate buffer, pH 7.0, for 1 hr.	+	+
Pepsin: 0.05 mg./cc. in 0.02 N hydrochloric acid for 5 min.	−	−
Control: 0.02 N hydrochloric acid for 5 min.	+	+
Trypsin: 2 mg./cc. in acetate buffer, pH 4.0, for 5 min.	−	−
Control: acetate buffer, pH 4.0, for 5 min.	+	+

* A plus sign indicates that there was staining. A minus sign indicates that there was little or no staining.

RESULTS IN CATARACTOUS LENS

EFFECTS OF X-RAYS

Ophthalmoscopic examination of Rabbits H-1 and H-2 shortly before their death showed that the irradiated lenses were, as a whole, translucent. There were, however, a large number of small bubbles in the posterior subcapsular region with streaks of haziness extending from these bubbles. There was a slight general haziness of the anterior subcapsular region. The changes were much more severe in H-2 than in H-1. The greater susceptibility of H-2 may have been due to the younger age (10 weeks, as compared with 3 years). Histologically, the capsule was markedly thickened and distorted. The cytoplasm of the epithelial cells was sparse and irregular, with indefinite boundaries between cells. There appeared to be an increase in the number of cells present as compared with normal. Changes in the lenticular fibers were limited to the subcapsular regions. In these areas the fibers were conspicuously distorted. Generally, they were markedly swollen, but some fibers were smaller than normal. In H-2, numerous large vacuoles were present (Figs. 2, 3, and 4).

9. Prepared by Dr. Milton Levine from bull testes.

10. Obtained from the Wellcome Research Laboratories, Beckenham, England.

11. Obtained from Armour and Company.

Histochemical Localization of Polysaccharide-Protein Complexes.—With the modified Hotchkiss procedure, the capsule stained intensely. It was considerably thicker than the normal capsule. In some areas there were accumulations of intensely staining homogeneous material, either directly beneath or a part of the capsule. We have assumed that this material was the inner part of the capsule.

Fig. 2.—Photomicrograph of cataractous lens from Rabbit H-1 after the tissue had been prepared by freezing and drying, sectioned, and stained by the periodic acid-leucofuchsin method. The transversely sectioned fibers are markedly distorted, and there are many fibers which stained intensely. × 585.

There was marked variation in the degree of staining of the abnormal lens fibers, the large fibers, in general, showing more intense staining (Fig. 2). In the region of the lenticular fibers there were numerous areas of very deep staining (Figs. 2

and 3). These areas probably represented degenerating fibers and seemed to be the earlier stages of the vacuoles seen in Figure 4. The cement substance appeared swollen (Fig. 2).

With toluidine blue, structures staining metachromatically in the normal lens stained the same way in the cataractous lens. In addition, some of the abnormal fibers stained metachromatically.

Solubility of the Polysaccharide-Protein Complexes.—Solubility studies were carried out on cataractous lenses in the same manner as that described for normal

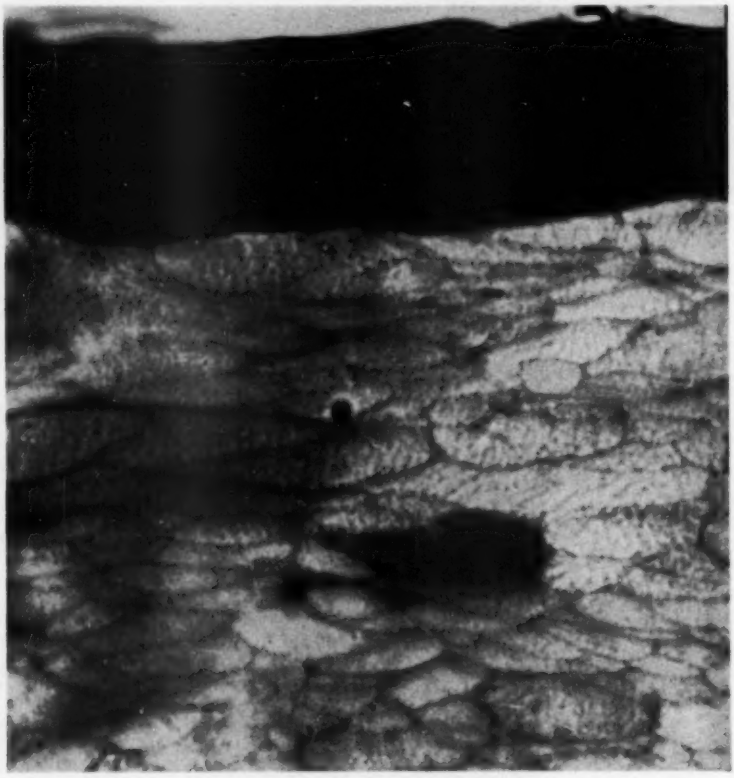

Fig. 3.—Photomicrograph of cataractous lens from Rabbit H-1 after the tissue had been prepared by freezing and drying, sectioned, and stained by the periodic acid-leucofuchsin method. The fibers are markedly distorted, and some have stained intensely. The cement substance is swollen as compared with the normal. The capsule is thickened. × 1,500.

lenses. The results are summarized in Table 1 *B*. It will be noted that the polysaccharide-protein complexes of the capsule increased in solubility, especially in the inner areas adjacent to the cortex of the lens. Whereas the cement substance was water and acid insoluble in the normal lens, it was completely soluble in the cataractous lens. The polysaccharide-protein complexes of the lenticular fibers remained water soluble, including the very dark staining areas of swollen fibers.

Enzyme Studies on the Carbohydrate-Protein Complexes.—Enzyme studies were carried out in the same manner as for normal lens. Only the outer part of the capsule stained in the control sections, since the remainder of the reactive material was soluble (Table 1 *B*). Therefore no statement can be made as to the action of the enzymes on any of the reactive material except that in the outer part of the capsule. This reacted in the same manner as that in the normal capsule.

Fig. 4.—Photomicrograph of cataractous lens from Rabbit H-2 after the specimen had been prepared by freezing and drying, sectioned, and stained by the periodic acid-leucofuchsin method. The fibers were cut longitudinally. Note the large vacuoles. × 580.

Protein Dilution.—The swelling of the fibers, capsule, and cement substance might have been the result of an increase in the fluid content of the lens. If this were so, there would be a decreased concentration of protein. Protein dilution was shown to be present by measurements made of absorption of ultraviolet light at 2,804 A. by lenticular fibers, the apparatus of Catchpole and Gersh [7] being used. At this wave

length, relative absorption, expressed as modified extinction values, should be directly related to protein concentration. Eleven normal fibers had values between 0.77 and 0.98, whereas the fibers of the cataractous lenses showed between 0.18 and 0.70 on 11 measurements, the low readings being characteristic of the large swollen fibers and the nearly normal readings being characteristic of the very small fibers. The high values were not numerous in the abnormal tissue.

Summary.—Three definite changes occurred in the structures containing the polysaccharide-protein complexes as a result of exposure to x-rays:

1. There was marked swelling of the capsule, the fibers, and the cement substance. In the fibers, the swelling was accompanied by an increase in fluid content.

2. There was an increased reaction with the Hotchkiss stain, especially marked in the large lenticular fibers but also present in the capsule and cement substance.

3. There was an increased solubility of the carbohydrate-protein complexes, especially in the capsule and cement substance.

Effects of Slow-Neutron Radiation

The damage to the lenses caused by slow-neutron radiation appeared much more marked in the six rabbits examined by us than the damage caused by x-rays. There was nearly complete opacity of the lens on ophthalmoscopic examination. The capsule and epithelium appeared the same as in the lenses exposed to x-rays. Changes in the lenticular fibers were limited to the subcapsular regions, but were much more extensive than after x-rays. In the affected regions the lens fibers were largely degenerate, with a loss of normal architecture. Because of the marked damage and relatively late stage of cataract formation, extensive studies were not carried out on slow-neutron-induced cataracts.

COMMENT

On the basis of the Hotchkiss technique and toluidine-blue staining for metachromasia in sections of frozen-dried specimens, polysaccharide-protein complexes were found to be present in the capsule, cement substance, cytoplasm of the subcapsular epithelial cells, and lenticular fibers of the normal lens. These findings are in keeping with the chemical analyses of Krause,[2] who has reported that the capsule is an "albuminoid substance containing an amino-carbohydrate group." He has also reported that 0.84% of the total protein of the decapsulated lens is "mucoprotein," containing 10% carbohydrate, which he suggests is "an extracellular protein holding the fibers together." It is interesting to note that Krause found a small amount of carbohydrate in all of the proteins of the lens, including alpha crystallin 0.5% and beta crystallin 0.1%, the chief constituents of the lenticular fibers. This observation, perhaps, explains the slight but uniform staining of the lenticular fibers with the Hotchkiss stain.

The polysaccharide-protein complexes of the normal lens were divided into two main groups on the basis of solubility: that in the capsule and cement substance, which is insoluble in water and hydrochloric acid down to a pH of 1.0, and that in the fibers and epithelial cells, which is water and acid soluble. The polysaccharide-protein complexes in the capsule and cement substance are probably the chief constituents of the ground substance of the lens. Following exposure to x-rays or slow

neutrons, the polysaccharide-protein material in the cement substance and inner part of the capsule became water and acid soluble. There were also swelling and increased reaction with Hotchkiss stain in these areas.

These changes, we believe, can best be explained on the basis of a depolymerization of the polysaccharide-protein complexes of the ground substance by the radiation. The depolymerization of high polymers such as the polysaccharide-protein complexes of the lens would be accompanied by increased swelling and solubility. The increased reaction with Hotchkiss stain can be explained, at least in part, on the basis of the freeing of reactive groups which takes place as a result of the depolymerization.

The generally accepted explanation of the pathogenesis of radiation cataracts is that the primary damage is in the actively proliferating cells of the anterior epithelium. These cells then produce abnormal fibers which, after a variable period, disintegrate, with the production of a subcapsular cataract. This view is shared by Poppe,[12] Rohrschneider,[13] Leinfelder and Kerr,[14] and Krause.[15] Poppe [12] has shown that, after a variable latent period, there is a marked proliferation of epithelial cells following radiation to the lens.

It is important to point out the role which the ground substance may have in the proliferation of the epithelial cells and growth of the abnormal lenticular fibers. Gersh and Catchpole [16] have shown that in the process of invading connective tissue, tumor cells, directly or indirectly, cause the ground substance to become depolymerized. Aterman [17] has demonstrated that at least one of the local factors involved in the regeneration of liver is the state of the ground substance, in that the character of the basement membrane is altered by becoming thicker, staining more deeply, and having a larger homogenous component. The same phenomenon has been demonstrated by Miller [18] in working with regeneration of striated muscle following atrophy from neural injury. Elchlepp [19] has shown that depolymerization of the orbital connective tissue can be correlated with periods of rapid growth of the eyeball in the chick embryo, and Moscona [20] has made the same observation on the growth of the limb bud in the chick embryo. To this list of instances of the importance of the softening of ground substance for growth should now be added the epithelial proliferation and growth of abnormal fibers in the lens following radiation injury.

It is possible that the depolymerization of the ground substance of the lens is due to the direct action of the radiation on the polysaccharide-protein complexes in a manner similar to the breakdown by radiation of polymers in vitro. It is also possible that the lens changes described may result indirectly from action of the radiation on the epithelial cells.

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13. Rohrschneider, W.: Strahlentherapie **31**:596, 1929.
14. Leinfelder, P. J., and Kerr, H. D.: Am. J. Ophth. **19**:739, 1936.
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19. Elchlepp, J. G.: Personal communication to the authors.
20. Moscona, A., and Moscona, H.: Personal communication to the authors.

CONCLUSIONS

Polysaccharide-protein complexes have been demonstrated in the capsule, cement substance, epithelial cells, and fibers of the normal lens.

Following irradiation of the lens, these changes take place: 1. There is swelling of the capsule, the lenticular fibers, and the cement substance. 2. There is an increased reaction with the Hotchkiss stain in these areas. 3. There is increased solubility of the polysaccharide-protein complexes in these areas. These changes are interpreted as resulting from a depolymerization of the polysaccharide-protein complexes of the lens.

The relation between the depolymerization and the pathogenesis of radiation cataracts has been discussed.

POSTMORTEM X-RAY STUDIES OF CONGENITAL MALFORMATIONS OF THE HEART

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INTRODUCTION

BY THE publication of our results we would reiterate the practical use of postmortem x-ray investigation of the vascular system following the injection of a contrast substance. Laubry and others have used this method for studying the topography of the heart and great vessels in the cadaver. Our experience has shown us that the procedure can be used post mortem to make a differential diagnosis in cases of cardiac and associated vascular malformations.

It was at the request of the clinicians that we undertook the investigation originally. Modern clinical and x-ray examinations have made possible the recognition of many more details of the heart in the living subject with far greater accuracy. These newer clinical x-ray investigations have given rise to many questions of identification, particularly of the form, shape, and topographical situation of the heart chambers and great vessels and their associated structures. We hoped to clarify some of the more difficult problems which beset the clinician.

It became clear to us during the making of films after a gradual filling of the cardiac and vascular systems that one could ascertain the abnormality present and, although the method of injection is completely different from that used in angiocardiography in the living patient, by comparing the postmortem films with the clinical films in many cases offer an explanation. It is possible to recognize the vessels providing a collateral blood supply, and when a collateral system exists filling of the fine ramifications may be watched while the injection is in progress. The method possesses an especial value for the future clinical diagnosis of similar cases.

A second advantage is predominantly anatomical. We were able to ascertain the abnormality in each case prior to preparation of the specimen and to make an exact diagnosis, thus enabling further investigation to be carried out on a rational basis from the start by indicating a general plan of dissection.

Supplementary to these advantages is the value of such films in teaching. In any demonstration of cardiac malformations, only a small number of students can be taught at one time and the specimen has to be fully dissected. The latter fact

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largely precludes use of the specimen for more detailed needs. Complete dissection calls for no mean measure of imagination on the part of the student, who is asked to reconstruct the anatomical and functional relationships between the various chambers and vessels in order to obtain a true picture of the blood flow in vivo. It has been our experience that students understand much more concerning cardiac malformations from a study of x-ray films than from a heart which has been opened in the classic manner. In other words, this method shows the abnormality in the abstract and facilitates reconstruction of the blood flow during life.

MATERIAL AND TECHNIQUE

The material, consisting of thoracic and sometimes of abdominal contents also, must be received fresh from the postmortem room, and no fixation may be undertaken until all the injections have been made and the films taken. It is better, moreover, not to disturb the organs any more than is absolutely necessary for an identification of the various blood vessels and the heart chambers. We have found no advantage in washing out the specimen.

In all the specimens ligatures are placed first on the main branches of the aorta at their cut peripheral ends in the root of the neck, on both the jugular veins or the superior vena cava, and on the azygos vein level with the commencement of the descending aorta. When the liver was not attached to the specimen the inferior vena cava was ligatured below the diaphragm or, in the absence of the diaphragm, distal to its entry into the right atrium. In the few cases in which the remaining portion of the vessel was too short, the opening in the wall of the right atrium was closed by suture. When the liver was present the inferior vena cava was ligatured, either above or below the organ according to the plan of injection. Later on, in order to prevent leakage from the numerous intercostal arteries, a ligature was also placed on the aorta at the junction of the arch and the descending portions. When it was desirable to maintain a clear lung field until the pulmonary artery was filled directly, an intrapericardial ligature was placed on the pulmonary veins; this was always a broad occluding ligature so that it might be removed later.

Injections of a suspension of barium sulfate in water were made into the great vessels or cavities of the heart, and an x-ray film was taken after each injection.

The injections were usually made with a wide-bore needle and a 20-cc. glass syringe; on occasion, however, it was found practical to replace the needle by a cannula or catheter, which was tied in position. The density of the barium suspension was always limited by the bore of the needle, but in general we found a suspension of 200 gm. of barium sulfate in 500 cc. of water satisfactory. Choice of site for the initial injection was influenced by the expected findings as judged from the clinical report. In the case of a possible interventricular septal defect a broad occluding ligature was placed around the heart in the atrioventricular grooves; this not only separated the atria and ventricles, but also closed the two main arteries. Unless this ligature was tied very tightly—a procedure which is damaging and seldom profitable—a small quantity of contrast material might leak into the ascending trunks. If an abnormality of the pulmonary artery was suspected, a filling of this system by means of a direct injection into the artery or one of its main branches might precede ligation. The posteroanterior position was found to show the origin of the pulmonary artery most adequately. The initial injection into each chamber might be complete or the contrast substance might be divided into two or more fractions, a film being taken after each was injected.

The following scheme for injection may generally be followed, and unless otherwise stated exposures are made with the specimen in the anteroposterior position.

1. A broad ligature is placed around the heart in the sulcus coronarius.
2. Contrast medium is injected into the right ventricle.
3. An occluding ligature is placed around the pulmonary hili.
4. Contrast medium is injected into the pulmonary artery (branch or stem) or truncus arteriosus as the case may be and a posteroanterior film taken.
5. If, after the film has been taken, the ductus arteriosus is seen to be patent or may be presumed to be normally still open, a ligature may be placed around the aorta proximal to the opening of the ductus.
6. The ligature in the sulcus coronarius is now removed, a further injection made into the pulmonary artery, and a film taken with the specimen in the posteroanterior position.
7. The right atrium is now filled directly and this procedure will demonstrate an atrial-septum defect when one is present unless, as occasionally happens with overfilled ventricles on the removal of the first ligature, a retrograde filling of the atria has occurred.
8. An injection is now made into the left ventricle, which will fill the associated vessel.
9. Finally, the left atrium is injected, the injection normally filling the pulmonary veins. These veins may have been lightly filled via an atrial-septum defect, but marked filling seldom occurs until the left atrium itself is injected.

An abnormality determined at any given stage may make it advisable to alter the order of this scheme, as will be seen in the following eight cases, chosen from our material to illustrate the method.

REPORT OF CASES

The sequence of the injections is given for all the cases in the legends to the accompanying illustrations. The diagrams were drawn from the corresponding films; to the initial diagram in each case the outlines of the successive fillings were added. The serial numbers in parentheses in the legends are those of the x-ray films in the Anatomical Institute at Leiden. Abbreviations used in the drawings are as follows: *V. D.*, ventriculus dexter; *V. S.*, ventriculus sinister; *V. I.*, ventriculus impar (monolocularis); *A. D.*, atrium dextrum; *A. S.*, atrium sinistrum; *A. C.*, atrium commune; *Ao.*, aorta; *A. P.*, arteria pulmonalis; *T. A.*, truncus arteriosus; *V. P.*, vena pulmonalis; *V. C. S.*, vena cava superior; *V. C. I.*, vena cava inferior; *A. C.*, arteria coronaria; *V. C.*, vena coronaria; *S. C.*, sinus coronarius; *S. V.*, septum ventriculorum; *S. A.*, septum atriorum; *V. def.*, defect in the septum ventriculorum; *A. def.*, defect in the septum atriorum; *D. art.*, ductus arteriosus; *P. st. dil.*, poststenotic dilatation; *Anast.*, anastomosis, and *Coll.*, collateral system.

Brief summaries of the clinical and postmortem histories and of the respective anatomical examinations follow in the text.[1]

CASE 1.—A boy aged 8 years admitted to hospital at the age of 5 years with cyanosis and dyspnea, had returned in a much-deteriorated condition. Clinically, right-sided hypertrophy was pronounced; the hilar shadows were small and the pulmonary window clear. The angiocardiogram showed a large right atrium with an abnormal form of the right ventricle, the lower border of which seemed to overlie the diaphragm. Simultaneous filling of the aorta and a narrow pulmonary artery was obtained. These vessels and the right heart remained filled for a long period. A small degree of overriding of the aorta was seen. A clinical diagnosis of

1. Cases 1, 2, and 6 have been more extensively described, especially in respect to the collateral vascularization of the lungs, in: Collister, R. M.: Collateral Circulation in Stenosis of the Great Vessels, Thesis, Leiden, 1952.

tetralogy of Fallot was made, which was confirmed by heart catheterization. The child underwent an operation for creation of a Blalock-Taussig anastomosis. He died on completion of the operative procedure.

The thoracic contents were placed at our disposal through the courtesy of Dr. H. Wamsteker of Haarlem, and the procedure of filling the heart chambers progressively with barium sulfate was carried out as described earlier in this article. Four films were chosen from the series and are illustrated in Figures 1 and *2 A*.

The postmortem x-ray diagnosis was a typical tetralogy of Fallot, and this was verified by anatomical dissection carried out after fixation of the material.

Case 2.—A boy aged *2* years and 3 months was admitted to the hospital with attacks of dypsnea and cyanosis. The angiocardiogram showed an open septum atriorum, with pronounced pulmonary stenosis. Overriding of the aorta could not be demonstrated. One year later the baby returned with a high fever, constant cyanosis, and slight dyspnea at rest. X-ray examination showed no process in the lungs to account for the raised temperature, which was not influenced by penicillin therapy. Heart catheterization was carried out. A clinical diagnosis of probable tetralogy of Fallot with an atrial-septum defect was made, but pulmonary stenosis with right-to-left atrial shunt was considered a possibility. Two months after admission to the hospital convulsions developed and the baby showed signs of shock and became very blue; he died two days later.

Immediately after the postmortem examination, performed in the Pathology Department at Leiden (Section 76, 1950), a progressive filling of the heart chambers with barium sulfate was carried out. From the series of films obtained three were chosen to illustrate the case Figs. *2, B* and *C*, and *3 A*).

The diagnosis made from the x-ray films was an open septum atriorum with stenosis of the pulmonary orifice and very marked poststenotic dilatation. The simultaneous filling of both ventricles because the left side had a patent connection with the right atrium was at first suggestive of an open septum ventriculorum.[2]

On dissection of the specimen later, the pulmonary stenosis was confirmed, as were the open septum atriorum and the completely intact septum ventriculorum. The stenosis was valvular, the valves being fused in a conical form.

Case 3.—Cyanosis was first noticed in a baby boy at 3½ months. It became worse during the next two months and dyspnea developed. At the ages of 7 months and 11 months the baby was admitted to the hospital, the second time with suspected pertussis infection. The dyspnea and cyanosis then were extreme and the venous congestion of the neck veins pronounced. The apex beat was on the right side of the sternum, and straight x-ray films gave the impression of a situs inversus; as the lung shadows were faint a stenosis of the pulmonary artery was considered probable, while the bulging of the esophagus in the anteroposterior position indicated a left-sided aorta. Treatment was unavailing, and the baby died within a month after the second admission to the hospital. No angiocardiography or catheterizaton had been carried out.

The thoracic contents with the sternum attached were received by us immediately after the postmortem examination from the Pathology Department of Leiden University (Section 93, 1950), and injections of a suspension of barium sulfate were carried out. From the series of films obtained, four were chosen and are illustrated in Figures 3, *B* and *C*, and 4, *A* and *B*.

From our films a postmortem diagnosis was made of tetralogy of Fallot with absence of the interventricular septum giving a monoventricular heart. On anatomical dissection the apex of the heart was found to be directed to the right side, the external shape of the ventricles tended to be ovoid, and the monoventricular condition was confirmed. The pulmonary artery was found to have a very small opening lying posterior to a wide aortic orifice, but in addition to these findings the atria were found to be connected by a septal opening about 1.5 cm. long.

Case 4.—A boy aged *2* days was admitted to the hospital with cyanosis of the hands and feet, edema, and vomiting; he was very ill. No murmurs were audible. X-ray examination showed left-ventricle enlargement, and the clinical diagnosis was transposition of the great

2. In a previous, preliminary communication presented at the First International Congress of Cardiology, Paris, 1950, we described the case as an instance of tetralogy of Fallot. As mentioned here, however, no ventricular-septum defect was actually present.

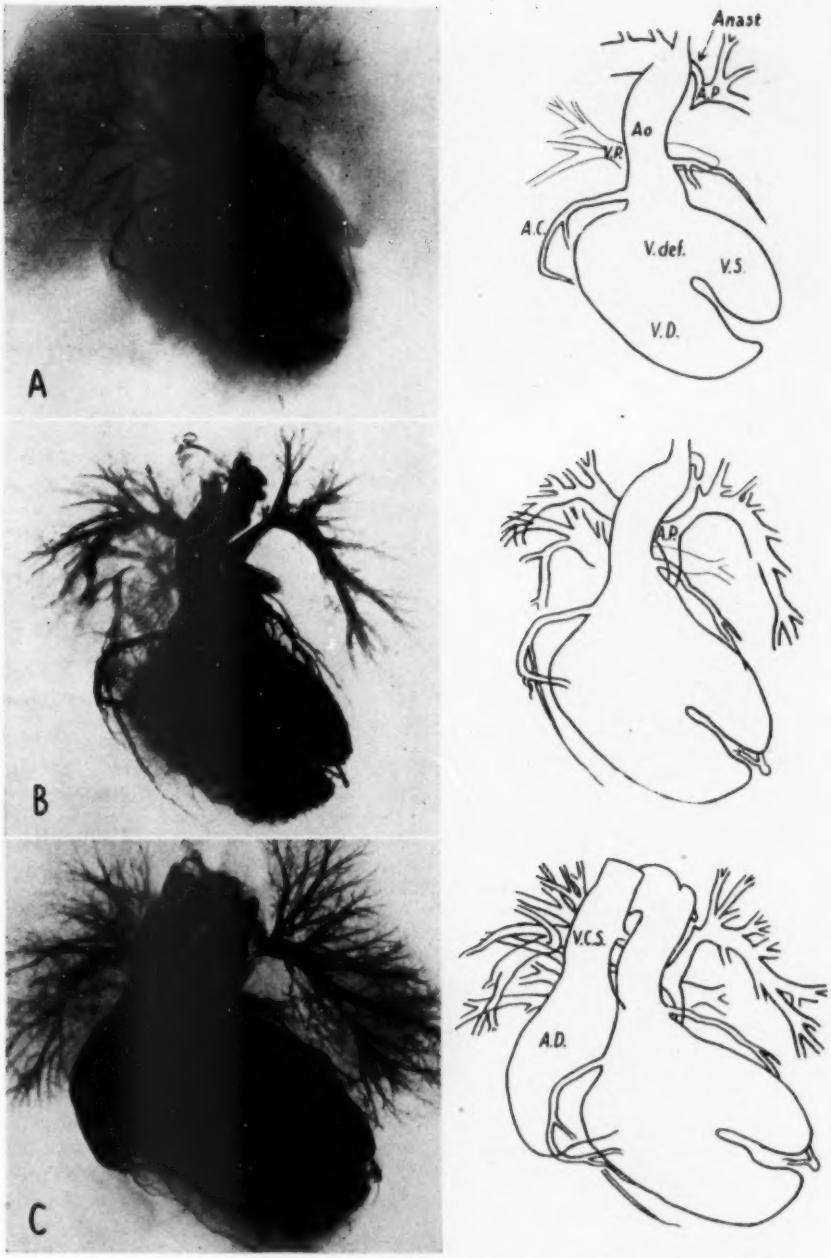


Fig. 1 (Case 1).—*A* (X-ray R 319), ligatures placed on the superior and inferior venae cavae and on the branches of the aorta; injection of 20 cc. of $BaSO_4$ into ventriculus dexter. Filling of both ventricles and aorta demonstrates a septal defect and overriding aorta. There is minimal filling of atrium sinistrum, right venae pulmonales, and arteriae coronariae. Slight filling of the vessel in the upper left lobe is due to patency of the operative anastomosis between the left subclavian artery and the left branch of arteria pulmonalis. *B* (X-ray R 320), ligatures placed on the anastomosis and at the origin of aorta descendens; injection of 40 cc. of $BaSO_4$ into ventriculus dexter. Filling of the ventricular cavities is more complete; the narrow arteria pulmonalis and branches have filled. More filling of atrium sinistrum and venae pulmonales to the left lung is now visible. No poststenotic dilatation of arteria pulmonalis is apparent. *C* (X-ray R 321), injection of 40 cc. of $BaSO_4$ into atrium dextrum. Filling of atrium dextrum and vena cava superior shows marked angulation of the atrium on the ventricle, thus emphasizing the thick wall of the ventriculus dexter. The pulmonary system has filled farther toward the periphery. Arcus aortae is superimposed on the upper part of the vena cava superior.

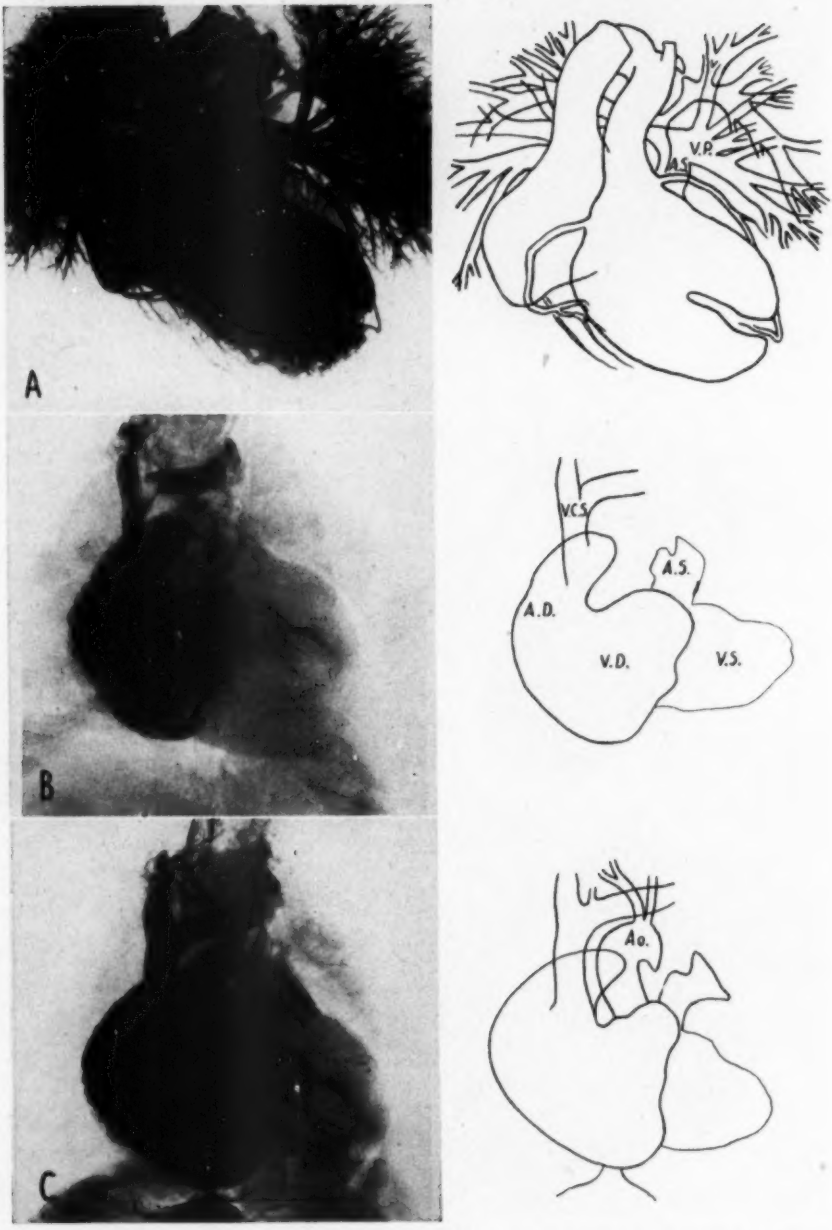


Fig. 2.—*A* (Case 1, X-ray R 322), injection of 40 cc. of $BaSO_4$ into atrium sinistrum. Filling of venae pulmonales to both lungs is now complete; the atrial contour can be seen. *B* (Case 2, X-ray R 395), ligatures placed on both venae cavae and on the branches of the aorta; venae pulmonales ligatured intrapericardially; injection of 40 cc. of $BaSO_4$ into ventriculus dexter. There are early filling of ventriculus dexter, more marked filling of a large atrium dextrum, and faint filling of atrium sinistrum, vena cava superior, and the innominate vein. The atrial wall is trabeculate and the wall of ventriculus dexter hypertrophied. *C* (Case 2, X-ray R 397), injection of 60 cc. of $BaSO_4$ into ventriculus dexter at the apex. There are more extensive filling of both atrial cavities and filling of ventriculus sinister and the aorta and its branches via the atria. Barium has leaked through the vena cava inferior to fill the hepatic system.

vessels. At the age of 15 months the baby was readmitted with attacks of extreme dyspnea and marked cyanosis. The thorax bulged posteriorly, and the heart was further enlarged to both sides. Clubbing of the fingers was now visible, and the edema had subsided. The boy died in one of the increasingly severe attacks of dyspnea. Angiocardiography and heart catheterization had not been carried out.

The heart, lungs, sternum, and liver were sent to us by the Pathology Department of Leiden University (Section 23, 1950). We made the routine injections of barium sulfate, and from subsequent x-ray films chose four examples which most clearly indicate the abnormalities present (Figs. 4*C* and 5).

The diagnosis made from the postmortem films was transposition of the great vessels, the aorta arising from the right ventricle and the pulmonary artery arising from the left ventricle. The systemic veins drained normally into the right atrium, and the venous return from the lung fields was to the left atrium. The conclusions were confirmed on anatomical dissection, which was carried out later. In addition, however, this procedure also revealed the existence of an interatrial septal defect, which had not been seen on either our films or those of the clinic. The opening was small and covered by a peculiar mesh-like membrane, which must almost have succeeded in retarding any cross current of blood between the atria. Mention may be made here that the presence of a double shunt would seem to be essential to maintain life; one possibility for providing a shunt was found in the atrial-septum defect, which probably had little, if any, functional effect. The other connection, commonly used as a shunt, the ductus Botalli, was not found patent in our x-rays, nor was it suspected to be patent clinically, but microscopical examination will be carried out to determine the degree of closure.

Case 5.—A boy aged 1 month was admitted with dyspnea and cyanosis dating from the age of 14 days. The first attack had lasted five minutes. The thorax was more developed on the right side. The apex beat was not palpable; the pulse was fast and scarcely palpable. The liver was very much enlarged and the spleen was palpable 1 cm. below the left costal margin. The clinical diagnosis was congenital vitium cordis. Straight x-ray examination revealed a very much enlarged heart, predominantly to the left. The large vessels appeared smaller than usual in the anteroposterior film and broader than usual in the lateral view. No other investigation was possible. The baby was in the hospital only two days before he died.

After the usual postmortem examination the heart, lungs, and diaphragm with the sternum attached were sent to us by the Pathology Department of Leiden University (Section 238, 1950). A suspension of barium sulfate was injected into the heart and great vessels, commencing with an injection into the trunk of the pulmonary artery. We chose three of the series of films to demonstrate the case (Fig. 6). The great vessels were seen to arise in their normal positions, and, far from being stenosed, the pulmonary artery appeared larger than the aorta; no septal defect could be demonstrated between the ventricles. The ductus arteriosus was patent. There was a gross septal defect between the atria.

Macroscopical dissection later revealed, indeed, a patent ductus arteriosus with an atrial-septum defect. It was seen, however, that this defect was almost completely covered by a flap-like valve arrangement the free edge of which lay approximated to the anterior margin of the foramen ovale. The superior vena cava was found to be somewhat narrower than usual. The right ventricle was more hypertrophied than the left and had a considerable capacity; the right atrium did not appear to be proportionately enlarged.

Case 6.—Cyanosis was noticed clinically on the evening of the day of birth, and the baby, a boy, spent the following month in an oxygen tent. He grew slowly and became progressively more cyanotic. He was admitted to the hospital at the age of 7 weeks; he was poorly developed and had severe cyanosis, a respiratory rate at rest of 80 per minute, and a pulse rate of 140 per minute. No murmurs were audible over the cardiac area. Both clinically and roentgenologically dextrocardia, with pulmonary atresia was thought to be present. The baby died at the age of 4½ months after an exploratory thoracotomy.

The thoracic contents were received by us through the courtesy of Dr. van Nieuwenhuizen and Dr. Brom (Utrecht). Routine injections of a suspension of barium sulfate in water were carried out. From the films obtained we chose three to illustrate the case (Fig. 7).

The ascending trunk of the aorta and its branches are seen on the first film, as well as a vessel which originated apparently in the aorta and passed to the left lung. On the second

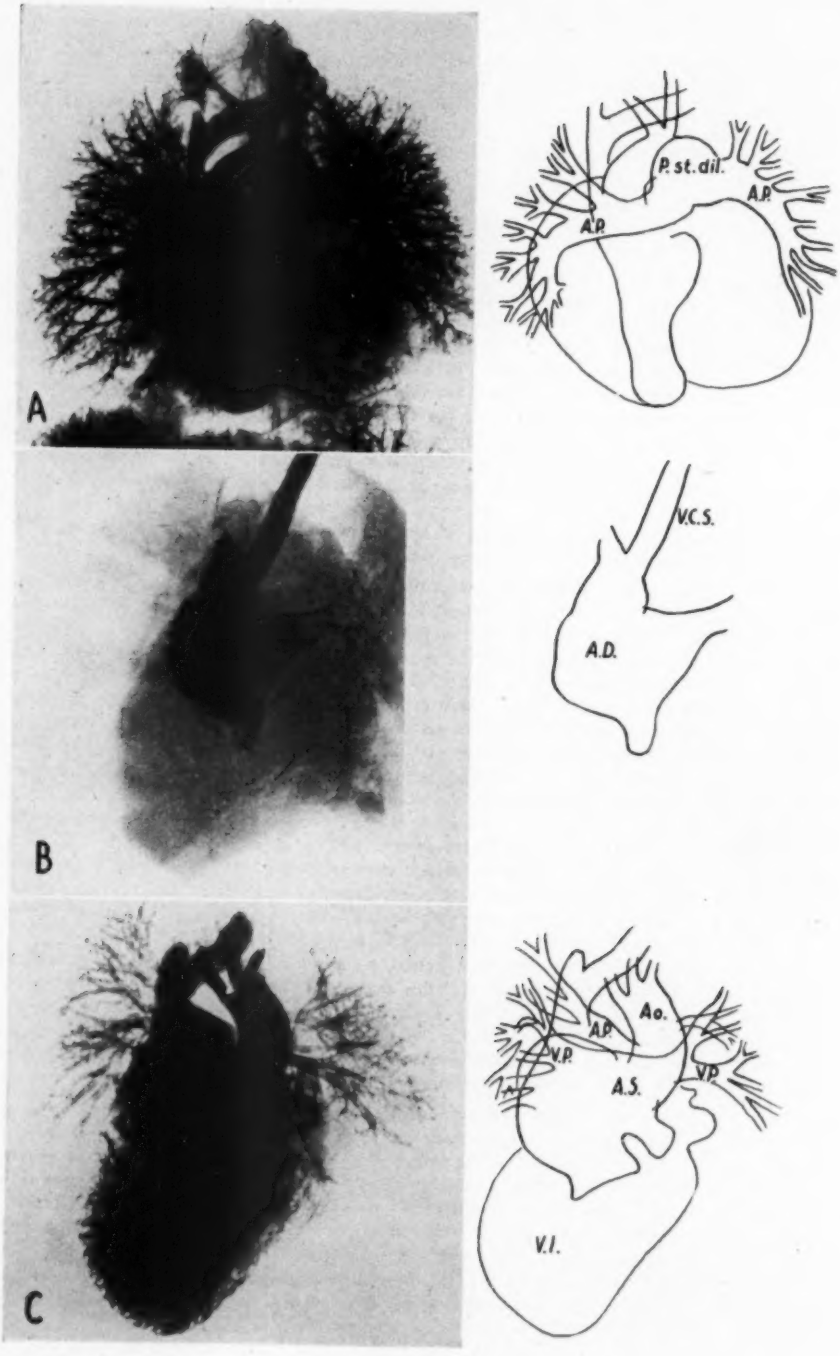


Figure 3

(See legend on opposite page)

film, made after injection of the arterial branch to the right lung in the lung hilum, there was obviously no connection between this vessel and either the heart or the aorta, but a surprising filling of collateral vessels lying on the medial surface of the right lung and in the mediastinum had occurred (Fig. 7 *B*). This system of collaterals was increased on filling of the bronchial arteries. The right atrium was enlarged, and there appeared to be a common ventricle. The pulmonary veins were not visibly filled.

Macroscopical dissection later confirmed the observation that the left lung supply arose in the aorta, that there was no connection between the main supply to the right lung and the ventricles of the heart, and that a large right atrium and a monoventricle were present. In addition, the foramen ovale was found patent about 3 mm., and the pulmonary veins drained normally to the left atrium. A widespread collateral network passing to the right lung was dissected macroscopically.

CASE 7.—A boy aged 3 days died of an undetermined congenital vitium cordis. He had been cyanotic from birth, and the blueness increased on crying. He was dyspneic with feeding. Clinically the heart was found to be enlarged to the left, and a loud harsh murmur was audible in the third intercostal space to the left of the sternum. The second sound was just audible. The liver extended 5 cm. below the right costal margin. No special cardiac investigation was possible in this case.

The specimen was received by us after the usual postmortem examination had been carried out by the Pathology Department of Leiden University (Section *227*, 1950). It consisted of heart, lungs, liver, and diaphragm. The sternum was absent.

The x-ray films after successive injections of barium sulfate into the heart and great vessels showed a single large ventricle, from which one vessel originated. As this vessel gave origin to both pulmonary arteries and was in a direct line with the descending aorta, a truncus arteriosus appeared to be present. The right atrium was filled both from the ventricle and with a direct injection, but the left atrium, with the pulmonary veins, could be seen only after a direct injection, which would indicate that there was probably no direct connection between it and the ventricle (Figs. 8 and 9 *A*).

On gross dissection the large right atrium and the single large ventricle were confirmed, and the apparent truncus arteriosus proved to be the pulmonary artery arising as a wide vessel from the right ventricle, giving the main branches to both lungs and leading into an arcus aortae. The latter connection must be interpreted as a widely persistent ductus arteriosus. The left ventricle was then found as a small appendage to the right chamber, and from this hypoplastic cavity a thin vessel took origin, the aorta, which passed upward on the left side of the pulmonary artery toward the neck; it connected with the descending aorta and in so doing also with the pulmonary artery.

The left atrium was very small, and although it received the pulmonary veins it did not connect in any way with the left ventricle; there was, moreover, no interventricular septal defect. One coronary artery took origin from the base of the small aorta and the other from the base of the pulmonary artery. The ductus arteriosus had the same diameter as the pulmonary artery and the aorta descendens, and the arrangement may be described, although not morphologically, as a functional truncus.

EXPLANATION OF FIGURE 3

Fig. 3.—*A* (Case 2, X-ray R 400), injection of 40 cc. of $BaSO_4$ into the trunk of arteria pulmonalis. There is filling of the stem and branches of arteria pulmonalis. Dilatation of this vessel is seen at point of bifurcation. No connection is seen between the pulmonary artery and right ventricle. The filling of atrium sinistrum is now less. In the absence of a direct filling of atrium sinistrum, venae pulmonales are not filled. *B* (Case 3, X-ray R 409), ligatures placed on both venae cavae, azygos vein, branches of the aorta, and commencement of aorta descendens; broad occluding ligature placed intrapericardially around the aorta and arteria pulmonalis; cannula placed in left innominate vein; injection of 25 cc. of $BaSO_4$ via the cannula. There are filling of atrium dextrum and some filling of atrium sinistrum and of the ventricular portion of the heart. *C* (Case 3, X-ray R 415), ligature around aorta and arteria pulmonalis removed; injection of 30 cc. of $BaSO_4$ through the wall of ventriculus dexter. There is filling of the common ventricle, wide aorta, and branches. The pulmonary arterial system is small, the right branch seeming larger than the left. Faint filling of venae pulmonales to the right lung is apparent.

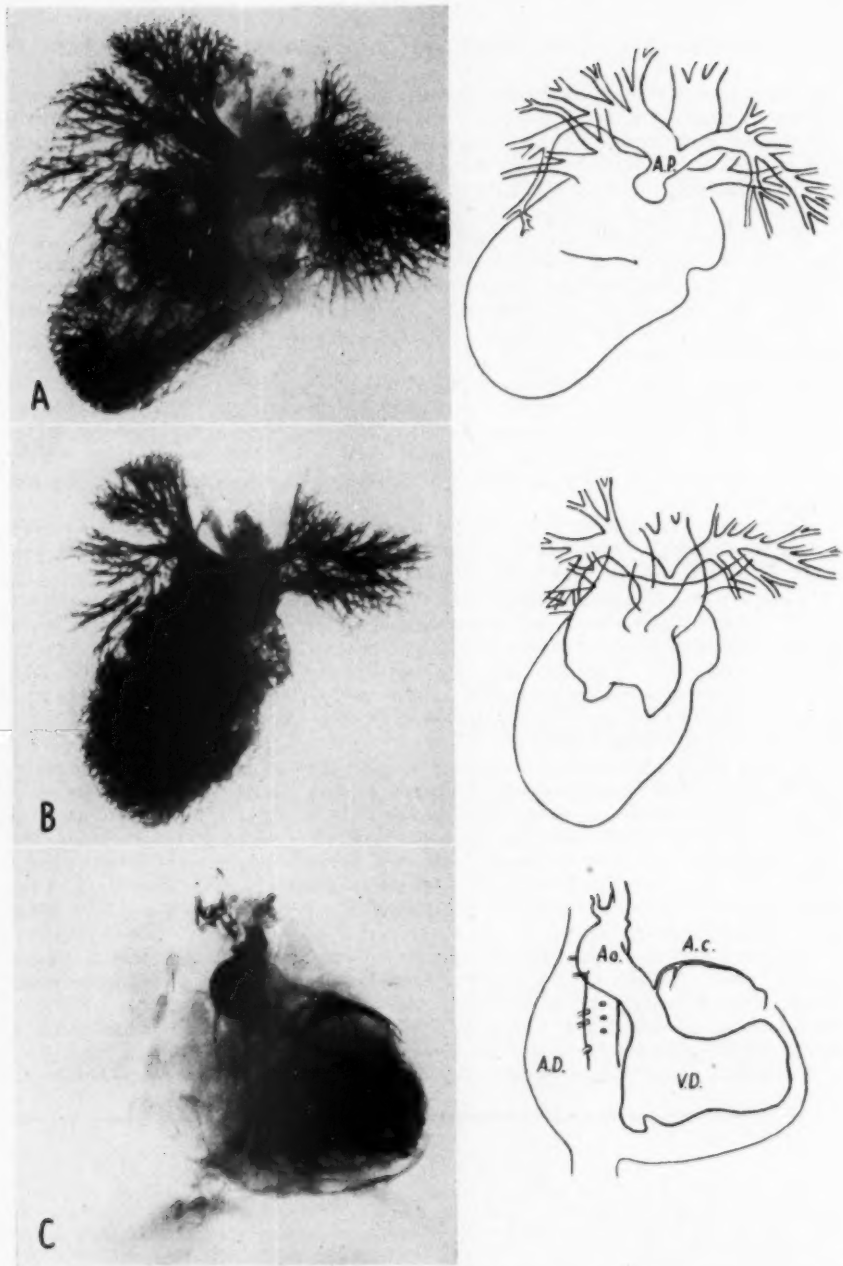
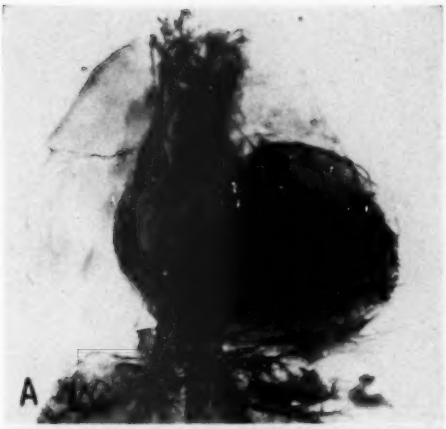
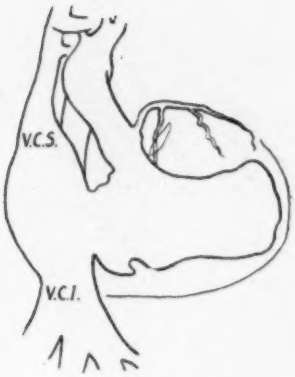
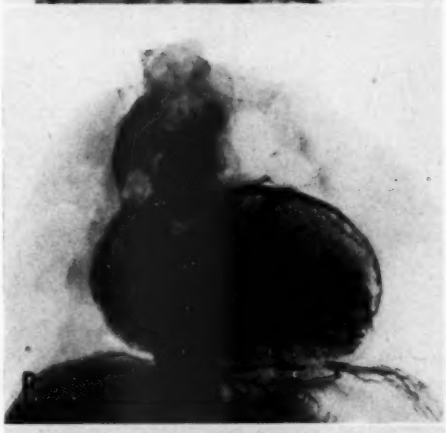
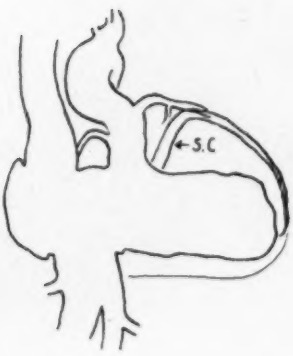


Fig. 4.—*A* (Case 3, X-ray R 418), heart emptied of barium by aspiration of ventricular portion and of atrium dextrum; injection of 40 cc. of $BaSO_4$ into the left branch of arteria pulmonalis. There is filling of arteria pulmonalis and branches. The right branch of arteria pulmonalis is seen to be much larger than the left branch. A common ventricular cavity is apparent, but no further filling of the heart chambers from arteria pulmonalis itself. *B* (Case 3, X-ray R 420), injection of 40 cc. of $BaSO_4$ into atrium sinistrum plus 16 cc. of $BaSO_4$ into the left branch of arteria pulmonalis. Filling of the heart cavities and aorta has recurred. Venae pulmonales are seen crossing the branches of the artery. *C* (Case 4, X-ray R 325), ligatures placed on vena cava superior and vena cava inferior below the liver; branches of the aorta also tied off, but not aorta descendens; injection of 10 cc. of $BaSO_4$ into ventriculus dexter. There is filling of ventriculus dexter, sinus coronarius, aorta ascendens, arcus aortae, aorta descendens, and arteriae coronariae.









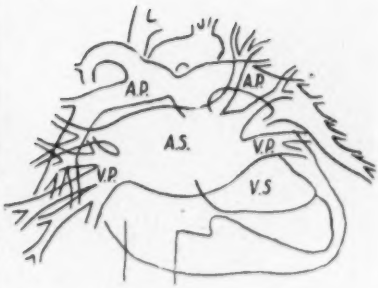


Fig. 5 (Case 4).—*A* (X-ray R 327), ligature not placed on beginning of aorta descendens; injection of a further 15 cc. of $BaSO_4$ into ventriculus dexter. There are further filling of ventriculus dexter and filling of atrium dextrum and venae cavae, the inferior showing as an abnormally broad vessel. *B* (X-ray R 329), injection of 14 cc. of $BaSO_4$ into aorta ascendens, with further filling of the aorta, right side of the heart, and sinus coronarius and a wing-like outline of arteriae coronariae. *C* (R 330), the right side of the heart washed out as fully as possible; injection of 15 cc. of $BaSO_4$ into ventriculus sinister. There is widespread filling of ventriculus sinister, arteria pulmonalis, atrium sinistrum, and venae pulmonales. No connection is present between the two ventricular cavities.

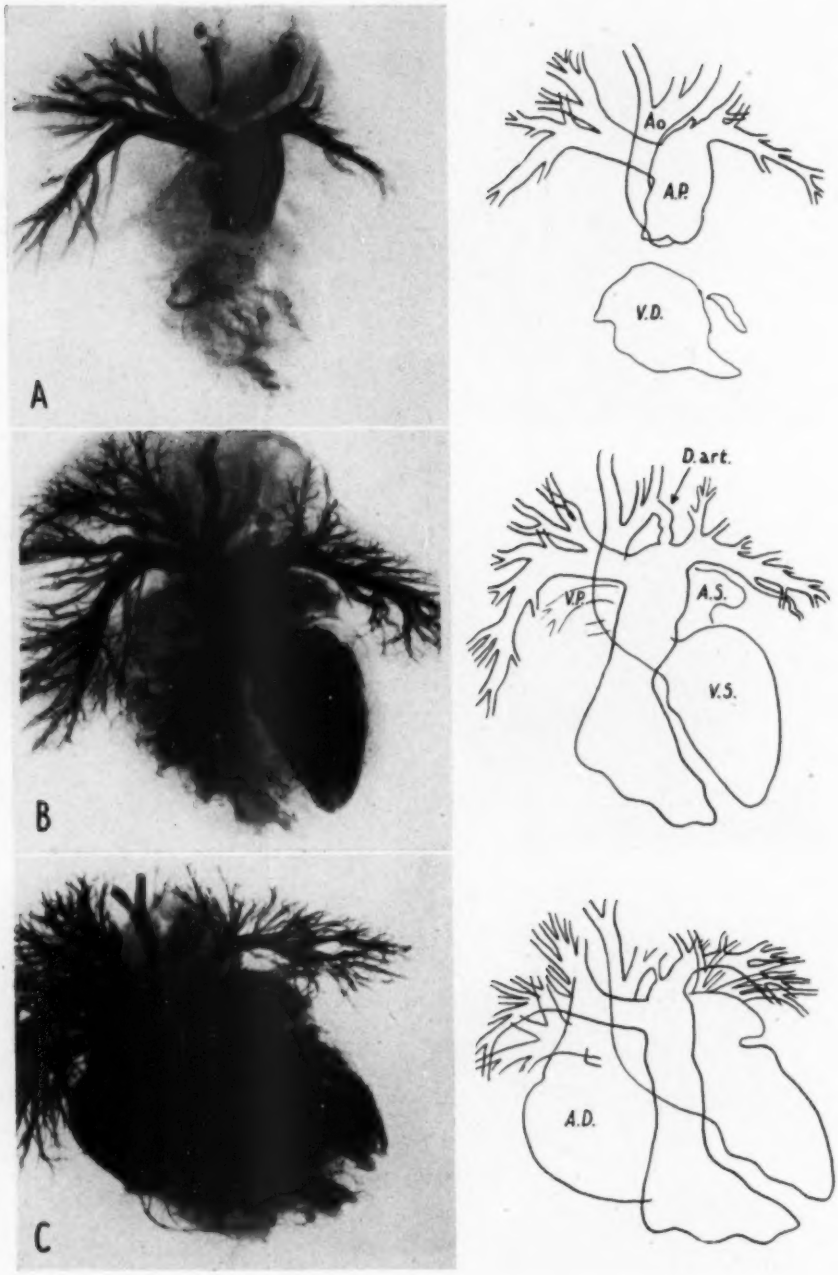


Fig. 6 (Case 5).—*A* (R 520), ligatures placed on venae cavae, branches of the aorta, and upper part of aorta descendens; injection of 12 cc. of $BaSO_4$ into arteria pulmonalis. There is filling of a wide arteria pulmonalis with branches in both lung fields. The valve outline is clear. Filling of aorta ascendens and branches shows this vessel to be smaller than arteria pulmonalis and indicates that ductus arteriosus is open. Filling of ventriculus dexter is shown. *B* (R 522), injection of 20 cc. of $BaSO_4$ into ventriculus sinister, with filling of ventriculus sinister and aorta and branches, including arteriae coronariae. Ductus arteriosus is seen widely patent, and further filling of the arteria pulmonalis system and ventriculus dexter has occurred. *C* (R 523), injection of 20 cc. of $BaSO_4$ into atrium sinistrum. Filling of both atria shows a large atrial-septum defect. Venae pulmonales are filled now, as is vena cava superior.

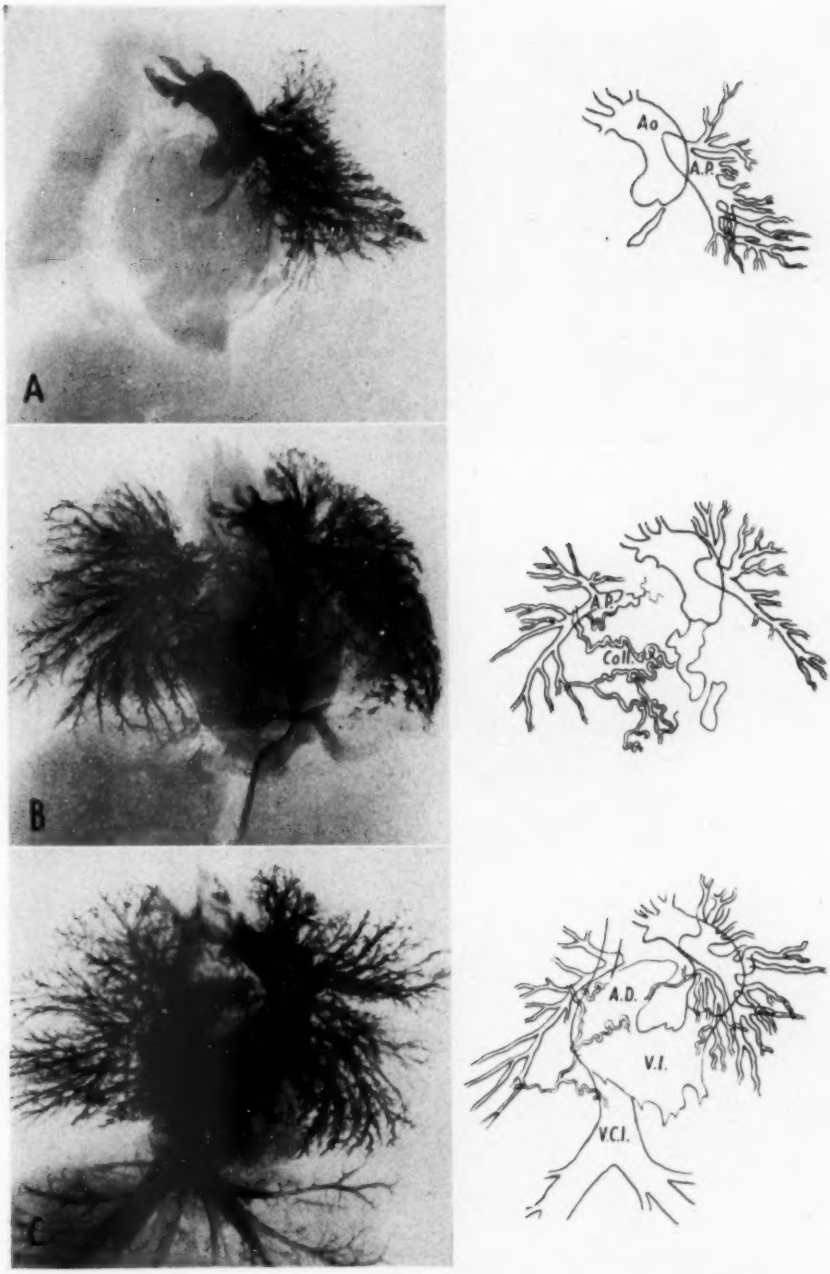


Fig. 7 (Case 6).—*A* (R 536), ligatures placed on vena cava superior and on the branches of the aorta; aorta descendens ligatured at its origin; injection of 6 cc. of $BaSO_4$ into the left branch of arteria pulmonalis. Filling of the aorta and its branches is shown, with filling of a branch passing to the left lung. There is no filling of vessels to the right lung or the heart chambers. *B* (R 537), injection of 12 cc. of $BaSO_4$ into the right superior branch of arteria pulmonalis, with filling of the arterial system in the right lung and of collateral vessels apparently between the lung and the surface of the pericardium. No connection between this branch of arteria pulmonalis and the heart or the aorta is apparent. *C* (R 544), injection of 10 cc. of $BaSO_4$ into ventriculus dexter, 4 cc. into atrium sinistrum, and 8 cc. into atrium dextrum, with filling of a ventricular cavity, outline filling of the atrial part of the heart, and filling of vena cava inferior and hepatic veins.

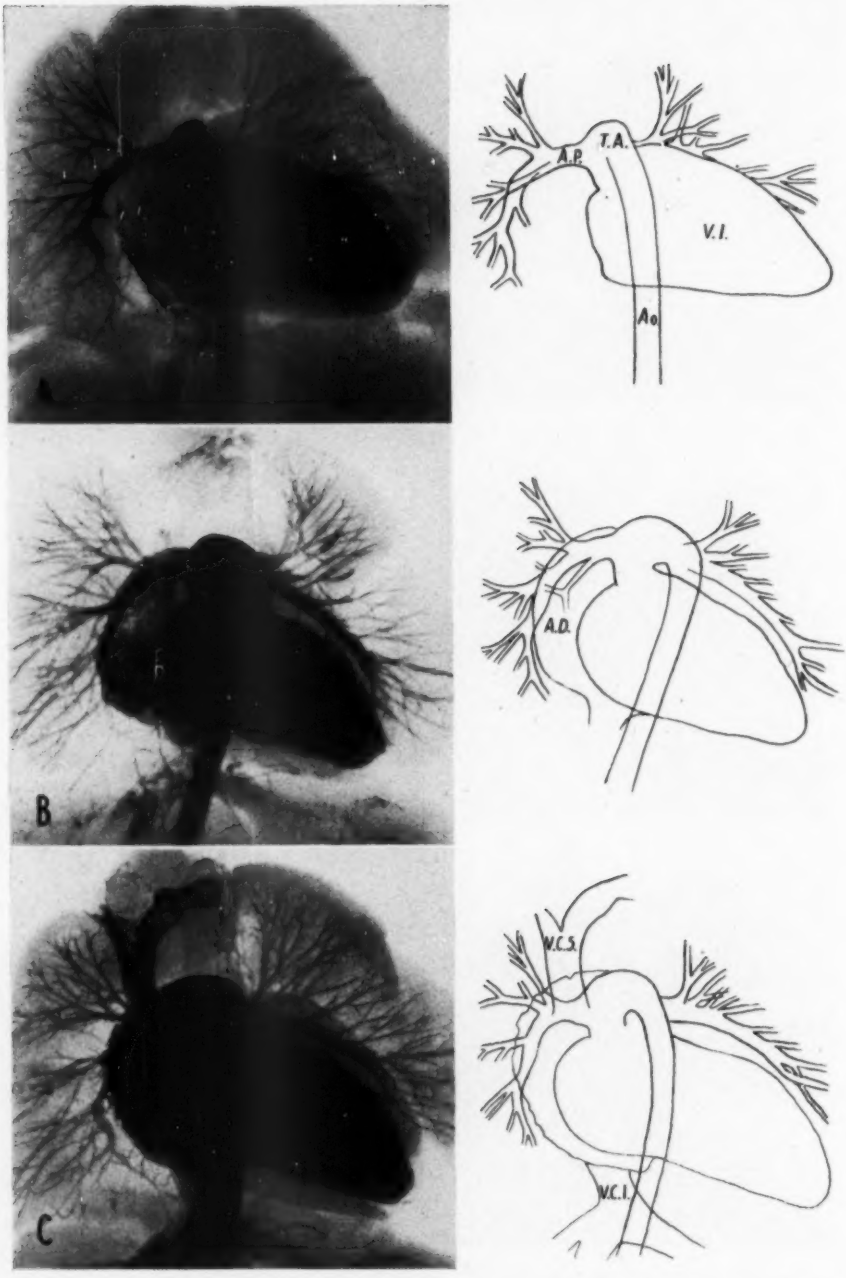


Fig. 8 (Case 7).—*A* (R 508), ligatures placed only on vena cava superior and branches of the aorta; injection of 13 cc. of $BaSO_4$ into truncus pulmonalis. There is filling of arteria pulmonalis, arcus aortae, aorta descendens (truncus arteriosus), and ventriculus impar, with slight filling of the right atrium and vena cava inferior. *B* (R 512), injection of 14 cc. of $BaSO_4$ into ventriculus. Ventriculus impar and atrium dextrum appear more filled, and the origin of truncus arteriosus is distinct. Branches from the aorta are absent. *C* (R 513), injection of 6 cc. of $BaSO_4$ into atrium dextrum, with filling of atrium dextrum and venae cavae. The heart is almost horizontal.

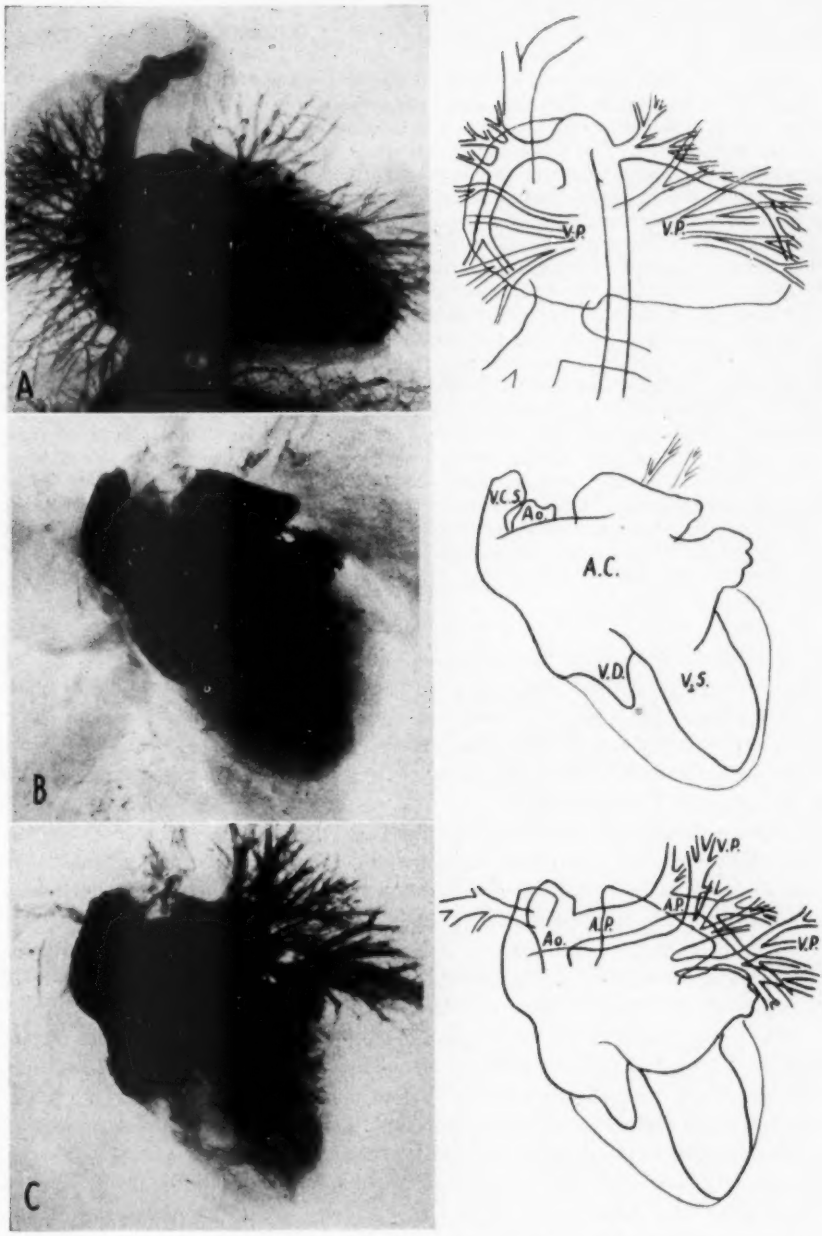


Fig. 9.—*A* (Case 7, X-ray R 515 *b*), injection of 10 cc. of $BaSO_4$ into atrium sinistrum, with filling of venae pulmonales but no clear outline of atrium sinistrum. *B* (Case 8, X-ray R 501), ligatures placed on venae cavae, branches of the aorta, and the lung hili; injection of 8 cc. of $BaSO_4$ into ventriculus sinister. There is filling of ventriculus sinister, of a small ventriculus dexter, and of both atria. Vena cava superior is seen filled on the right side; aorta ascendens lies to the left of it and both auricles to the left of aorta ascendens. Slight filling of arteria pulmonalis to the upper lobe of the left lung is apparent. *C* (Case 8, X-ray R 502), removal of ligatures around the lung hili; injection of 15 cc. of $BaSO_4$ into arteria pulmonalis. There is filling of both arterial and venous fields in the left lung, of arterial branches to the single-lobed right lung, and of the trunk of arteria pulmonalis. The atrial-septum defect appears to be large.

CASE 8.—At birth the patient, a boy, was placed in the premature unit because of obvious cyanosis. An intermittent short dry cough was present from birth, and from time to time there was an attack of increased cyanosis and dyspnea. He was admitted to the hospital at the age of 8 months. The fingers and toes were not clubbed. Straight x-rays showed an enlarged heart. Neither angiocardiography nor catheterization was carried out; the congenital vitium cordis remained unelucidated, although it was thought to be atresia of the tricuspid valve. In spite of treatment the condition became worse, and the baby died on the eighth day after admission. After postmortem examination the heart, lungs, and liver, with the diaphragm and sternum attached, were received by us from the Pathology Department of Leiden University (Section 165, 1950), and the usual x-ray investigation was carried out. Two of the films were chosen to demonstrate this case (Fig. 9, *B* and *C*).

It was possible to conclude from the postmortem films that a small right ventricle, a large left ventricle, and an atrium commune, with both auricles lying on the left side of the aorta, were present. As injections were first made into the right ventricle, filling the left ventricle and the atrium commune, it could not be determined from the films whether atresia of the tricuspid valve was present. Had this in fact existed, then the filling of the left ventricle following injection into the right ventricle could be explained only by the presence of an interventricular septal defect. Later, dissection disclosed a very large interatrial septal defect, giving a common atrium. The aorta arose normally from the left ventricle; the right ventricle was found to be an extremely small cavity from which a narrow pulmonary artery took origin. The tricuspid valve was atretic, and the right ventricle had a small, high connection with the left ventricle. The ductus arteriosus was patent only ± 1 mm.

SUMMARY

We have presented an introductory outline of a new method for investigating congenital cardiac malformations post mortem which involves a succession of injections into the heart and great vessels of a suspension of barium sulfate in water. An x-ray film is taken after each injection. The aim is always to make an exact diagnosis of the malformation present before any fixation or anatomical preparation of the specimen is undertaken.

The routine plan of injections is subject to alteration in the individual case, particularly when there has been no exact clinical investigation and diagnosis. In such cases, after the results of each injection have been studied on the subsequent film the procedure is modified appropriately.

Eight cases are reported, each of which demonstrates one or more salient points in the use of the method and its results, while some of the difficulties encountered in the interpretation of the films are also brought forward.

The cases presented include a typical case of tetralogy of Fallot; a case of pulmonary stenosis and patent foramen ovale; an instance of cor triloculare biatriatum with stenosis of the pulmonary artery; a case of transposition of the great vessels; a case in which the pulmonary artery was abnormally wide and the ductus arteriosus patent; an instance of atresia of the pulmonary artery with a single ventricle; a case of extreme stenosis of the aorta with a rudimentary left ventricle and a widely patent ductus arteriosus, which functionally was a truncus arteriosus, and a case of tricuspid-valve atresia with an atrium commune and a ventricular-septum defect, in which both auricles lay on the left side of the main arterial trunks.

The method and the illustrative cases are offered as an aid to preparation of such material, to systemic teaching, and also to the clinician, for the films help to elucidate the more obscure clinical cases and often serve as a basis for diagnosis of similar cases in the future.

HYPERVITAMINOSIS A IN YOUNG DUCKS

The Epiphyseal Cartilages

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AND
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THE PURPOSE of this report is to record the effect of excessive doses of vitamin A in young ducks upon epiphyseal cartilage, an effect similar to that recorded by us for the chick [1] and for several mammalian species.[2]

Experimental Data

Age, Days	Days on Diet *	Weights, Gm. Concurrent Controls No. 3	No. 4	No. 5	Weights, Gm. Hypervitaminosis-A Ducks No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Daily Vitamin A Dose (I. U.)
3	1	90	90	100	100	85	85	90	95	100	100,000
6	3	136	130	150	140	140	126	136	142	142	100,000
8	5	195	180	214	190	190	170	188	188	192	250,000
10	7	272	240	306	265	250	233	330	260	255	250,000
11	8	325	298	384	300	310	290	305	315	325	300,000
12	9	390	350	460	345	370	320	345	360	366	333,000
13	10	425	380	520	390	400	350	360	400	400	333,000
14	11	500	450	600	370	440	386	400	430	420	600,000
15	12	550	485	640	380	485	395	410	450	440	600,000
16	13	620	570	750	?	?	?	?	?	?	600,000
17	14	Killed	Killed	Killed	450	580	490	540	540	560	600,000
19	16	...	...	...	Killed	660	Killed	Killed	640	640	500,000
20	17	...	...	...	...	640	...	...	588	550	500,000
21	18	...	...	...	...	620	...	...	520	508	250,000
22	19	...	...	...	...	550	...	...	490	480	.....
..	..	...	...	...	...	Killed	...	...	Killed	Killed	.....

* Ducks were three days old when started on the diet.

The only published study of hypervitaminosis A in the duck is that of Rigdon, Rude, and Bieri,[3] who failed to find any change in the bones as a result of the administration of "large amounts of vitamin A."

Our wholly contradictory results may be explained in part by the facts that we gave attention to the epiphyseal cartilage sequences concerned in advancing endo-

This study was aided by research grants from The Nutrition Foundation, the Williams-Waterman Fund for the Combat of Dietary Disease, and Swift and Company, Chicago.

From the Division of Nutritional Research, Division of Laboratories and Research of the Children's Hospital; the Department of Nutrition, Harvard School of Public Health, and the Department of Biological Chemistry, Harvard Medical School.

1. Wolbach, S. B., and Hegsted, D. M.: Hypervitaminosis A and the Skeleton of Growing Chicks, A. M. A. Arch. Path. **54**:30, 1952.

2. Wolbach, S. B.: Vitamin A Deficiency and Excess in Relation to Skeletal Growth, Proct. Inst. Med. Chicago **16**:118, 1946; J. Bone & Joint Surg. **29**:171, 1947. Maddock, C. L.; Wolbach, S. B., and Maddock, S.: Hypervitaminosis A in the Dog, J. Nutrition **39**:117, 1947.

3. Rigdon, R. H.; Rude, J. C., and Bieri, J. G.: Effect of Hypervitaminosis A and Hypovitaminosis A on the Skeleton of a Duck, A. M. A. Arch. Path. **52**:299, 1951.

chondral bone growth and to texture of bone, particularly the resorption of bone and compact bone formation at sites of remodeling.

EXPERIMENTAL

The accompanying table contains pertinent experimental data for the control ducks and those which received excessive amounts of vitamin A. The diet was that used by us in our study of vitamin A deficiency in the duck [4] with the addition of

Fig. 1.—Normal Control Duck 3; distal epiphysis of the femur; × 8.

an adequate amount of vitamin A acetate for the controls or excessive amounts as shown in the table.

No gross changes other than the slower growth rate as indicated by the weights shown in the table were noted. No skin lesions were observed. The intake of food decreased as the experiment progressed—a usual effect of hypervitaminosis A.

4. Wolbach, S. B., and Hegsted, D. M.: Vitamin A Deficiency in the Duck: Skeletal Growth and the Central Nervous System, A. M. A. Arch. Path., to be published.

MICROSCOPIC PATHOLOGY [5]

Studies were made on a few bones only, as past experiences have shown that hypervitaminosis A acts upon endochondral growth of all bones. The more rapid the normal growth rate of various bones, the more conspicuous are the results.

Selected for study were the bones of the knee joint, distal femur, proximal tibiotarsus, lumbosacral vertebrae, and bones of cartilaginous origin of the skull.

Fig. 2.—Hypervitaminosis Duck 6; distal epiphysis of the femur; × 8. Note the absence of demarcation between zones of proliferation and growth. Note also the almost-complete consumption of mature (hypertrophied) cartilage cells and narrowed metaphyseal zone.

The Long Bones.—Figures 1 and 2, respectively the distal femoral epiphysis of Normal Control Duck 3 and that of Hypervitaminosis-A Duck 6, are representative

5. All descriptions were made from formalin-fixed bones embedded in celloidin and stained with hematoxylin and eosin.

of their kind. These photomicrographs may be compared with those of another normal control duck (Fig. 5) and a restricted-diet control duck (Fig. 6) of a previous publication.[4]

The outstanding features of the epiphyseal cartilage of long bones in the hypervitaminosis-A duck were a narrow zone of proliferating cells, a relatively wide zone of growing cells, no clear demarcation between zones of proliferation and growth,

Fig. 3.—Higher power detail of Figure 2; × 40. Note the advanced tunneling into the epiphyseal cartilage and concurrent resorption of tunnel walls in progress close to the advancing tunnels. Compare with the normal—Figure 1—in which, in spite of the low power, the normal lag in resorption of the tunnel walls is apparent.

and a very narrow zone of maturing and matured cells which was deeply penetrated by the tunnels characteristic of endochondral bone growth in birds. As in endochondral bone growth in hypervitaminosis-A chicks, the blood vessels were engorged and of larger caliber than in normal and restricted-diet control ducks. The epiphyseal cartilages at first glance might seem to have increased in width because of the wide

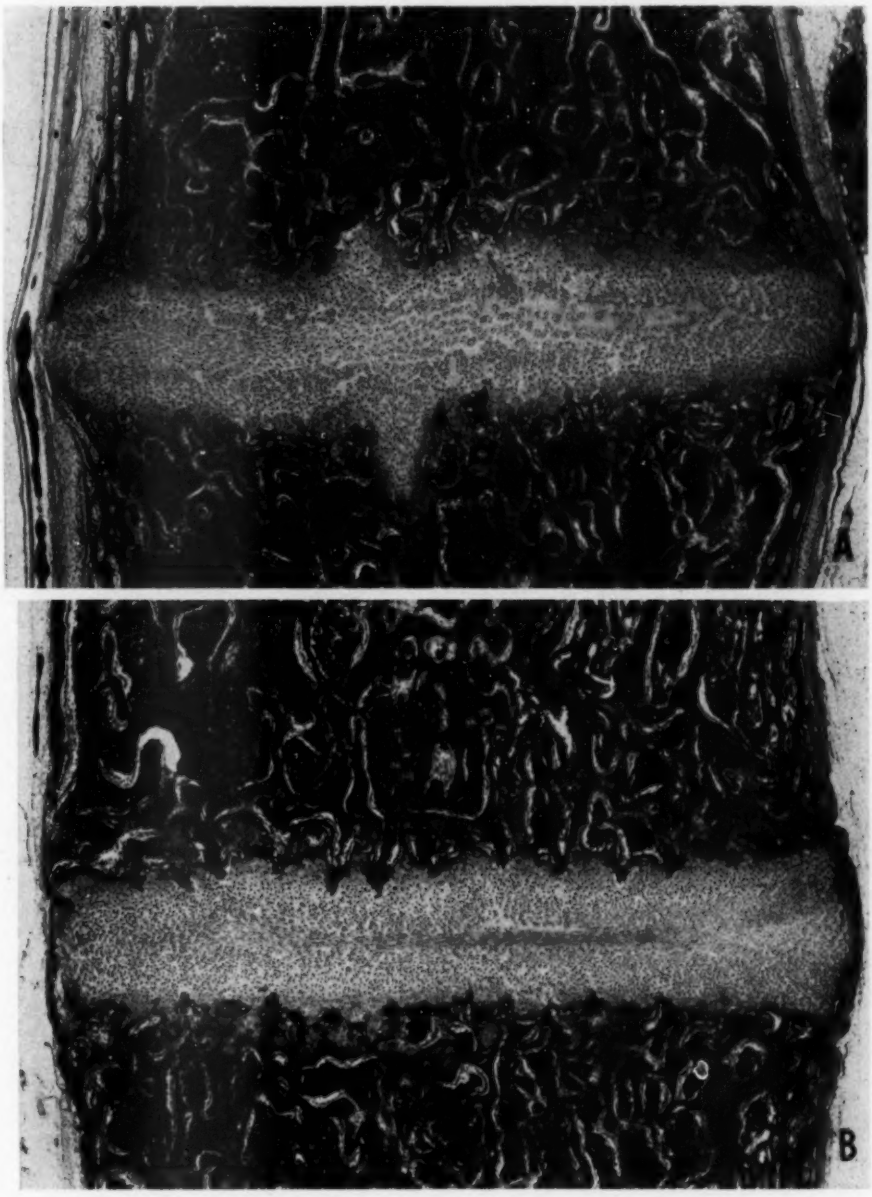

Fig. 4.—*A*, Normal Control Duck 3; lumbar vertebrae; × 40. This photomicrograph shows the appearance of normal endochondral bone growth of the lower vertebrae. *B*, Hypervitaminosis-A Duck 2; the same region as that shown in *A*; × 40. Note the narrowing of the epiphyseal cartilage and more prominent ingrowth of blood vessels.

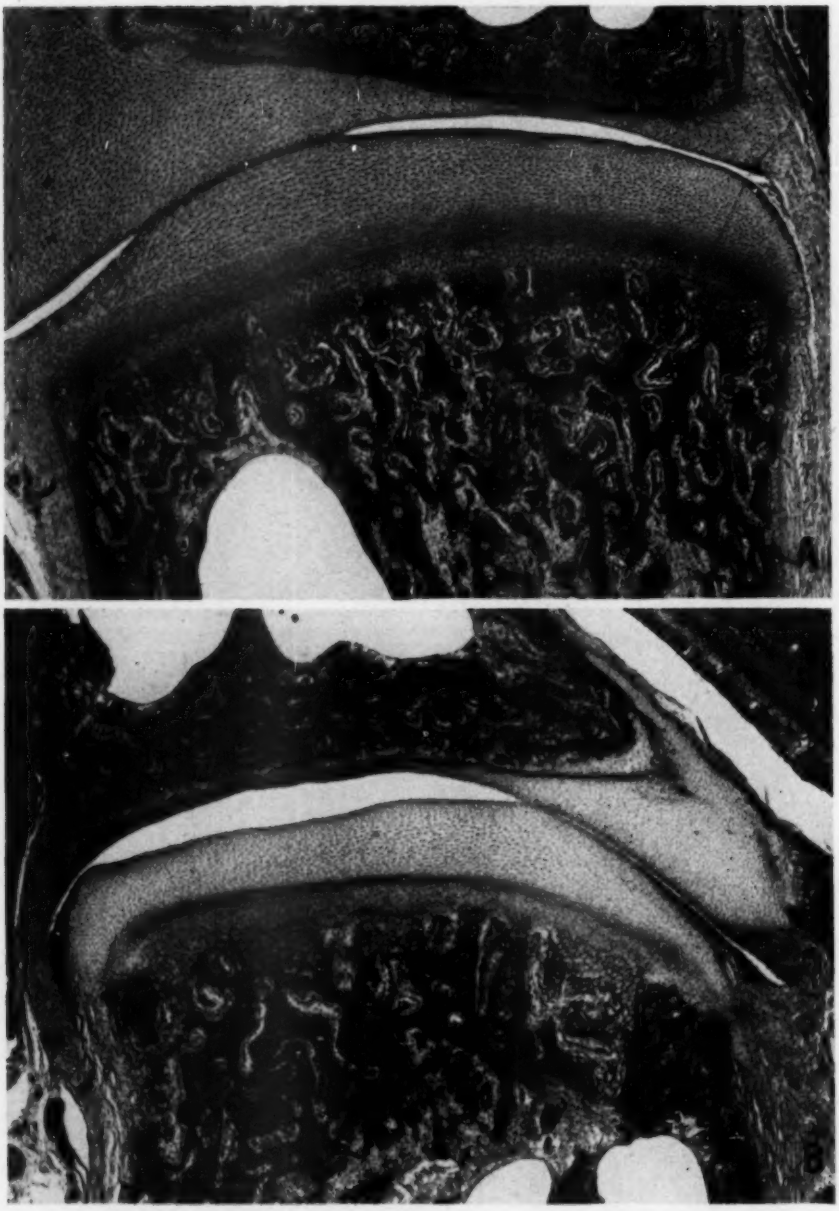

Fig. 5.—*A*, Normal Control Duck 4; frontal-plane section through the articulation of the caudal extremity of the mandible with the quadrate bone; × 40. Both of these structures are of endochondral bone growth origin. *B*, comparable section from Hypervitaminosis-A Duck 6; × 40. Note the narrowing of the epiphyseal cartilages of both bones and the denser trabecular bone.

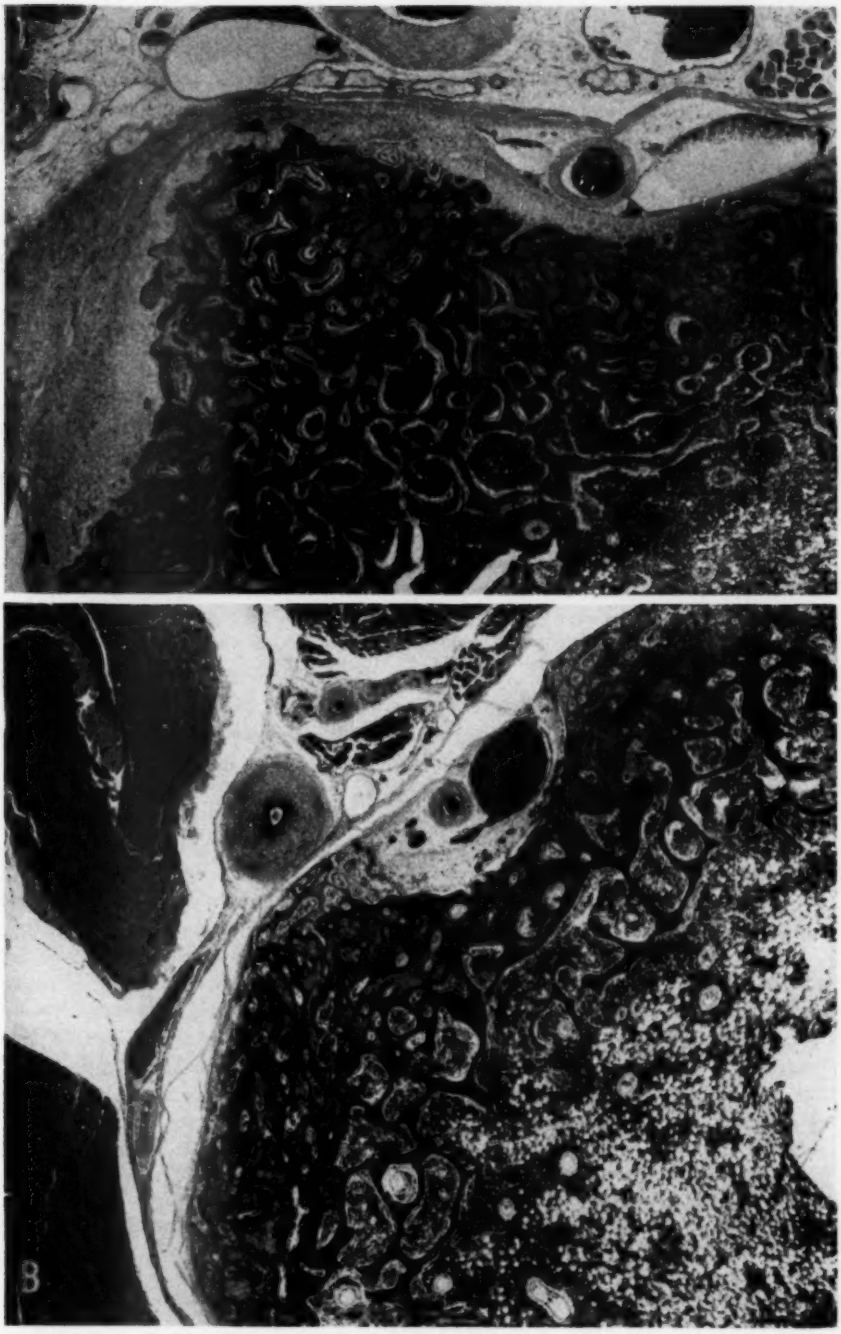

Fig. 6.—*A*, Normal Control Duck 3; cross section of the tibiotarsus; × 40. *B*, cross section of the tibia, Hypervitaminosis-A Duck 6; × 40. This section was taken at a slightly different level than that of *A*. It illustrates the same region of remodeling. Note the denser but thinner cortical bone.

zones of growing cells. Inspection of Figures 1 and 3 shows, however, that the tunneling has extended to the extreme limit of matured (so-called hypertrophic) cartilage cells. The combined width of epiphyseal cartilage and metaphyseal zones was decidedly less than that of normal controls (Fig. 1). Another evidence of accelerated endochondral ossification was resorption of the walls of the tunnels far in advance of that which normally occurs. Figure 3 shows the lateral resorption of tunnel walls close to the most advanced region of tunneling. Concurrently there was deposition of osteoid at the same high level of resorption of cartilage. The histological details were those of wider zones of chondrolysis. The cortical bone did not show the same degree of increased density resulting from accelerated compact bone formation that we found in chicks.[1] Figure 6, from cross sections of a hypervitaminosis-A duck and of a normal control at approximately the same levels, shows the thinner and denser cortical bone of the former.

The Axial Skeleton.—Endochondral ossification in the bones of the base of the skull, in the vertebrae, and in the mandible (Figs. 4 and 5), while less evident, presumably because of slower growth rates, showed the same features of acceleration that characterized the growth at the ends of long bones.

The Cortical Bone.—The cortical bone of vertebrae and skull showed much less increase in density (compact bone formation) than we found in the chick,[1] though differences became apparent when the bones were compared with those of normal and diet-restricted controls.

COMMENT

This article, the final one of a series dealing with the avian skeletal responses to vitamin A deficiency and excess, is presented without enthusiasm, solely for the purpose of showing that avian bone growth responds as does that of mammals when subjected to the same vitamin A dietary regimens.

Because the responses to hypervitaminosis A, like the pattern of fetal bone growth, are unrelated to the concurrent functions of the skeleton, and because postnatal bone growth is a continuation of fetal growth, we repeat our conviction that vitamin A has properties like those of inductor or evocator agents.

Thus far no organ or tissue has been shown to act as an intermediator. At present the anterior pituitary and probably the adrenal glands can be excluded.[6]

The remarkable responses described by Fell and Mellanby when vitamin A was added to embryonic bones of mice and chicks in tissue cultures,[7] and the effect of vitamin A upon the differentiation and histological structure of epitheliums derived from the chick embryonic ectoderm grown in tissue cultures characterized by suppression of differentiation into keratinizing epithelium and the substitution of a mucus-secreting ciliated epithelium,[8] almost compel the conclusion that vitamin A acts directly upon competent cells.

CONCLUSIONS

The growing skeletons of young ducks respond to excessive vitamin A administration precisely as do those of chicks and mammals. The only differences are quantitative and related to differences in rate of growth of various bones.

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6. Wolbach, S. B., and Maddock, C. L.: Vitamin-A Acceleration of Bone Growth Sequences in Hypophysectomized Rats, A. M. A. Arch. Path. **53**:273, 1952.

7. Fell, H. B., and Mellanby, E.: The Effect of Hypervitaminosis A on Embryonic Limb Bones Cultivated in Vitro, J. Physiol. **116**:320, 1952.

8. Fell, H. B., and Mellanby, E.: Personal communication to the authors.

NUCLEAR CHANGES IN RESPONSE TO CONTINUOUS IRRADIATION

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EXPOSURE of epithelial surfaces to ionizing irradiation from x-ray sources as well as from radioactive isotopes has long been known to be characterized by formation of enlarged hyperchromatic nuclei. These nuclei may be lobulated and contain discrete aggregates (chromatin) which stain intensely with routine histological stains.[1] Underlying these changes in nuclear morphology and staining qualities may be alterations in the chemical and physical characteristics of the nucleoprotein.

In the work to be discussed, nuclear aberrations produced by continuous irradiation from radioactive phosphorus were studied by means of histochemical procedures for the visualization of desoxyribonucleic acid. These methods were used in conjunction with fixation by freezing and drying.

The results consistently suggested that this type of irradiation may have an effect on the organization of nucleic acid complexes in vivo, possibly resulting in some degree of disaggregation.

METHODS

Fifteen young adult C57 mice were injected in the tongue with approximately 0.02 cc. of a suspension of radioactive chromic phosphate containing P^{32} (activity 800 μc per cubic centimeter).[2] Continuous irradiation from this beta ray source has previously been shown to result in the formation of numerous cells with atypical nuclei in the area of the injected mucosa.[1c] As controls some animals were untreated and others received an injection of nonradioactive chromic phosphate in the tongue.

Seven days after injection the tongues of both experimental and control animals were removed and fixed by freezing and drying.[3] The tissues were infiltrated and embedded in

Aided in part by grants from the National Cancer Institute, Federal Security Agency; the Department of the Army, and facilities of the Aeromedical and Physical Environment Laboratories, University of Illinois.

1. (*a*) Bloom, W.: Histopathology of Irradiation from External and Internal Sources, New York, McGraw-Hill Book Company, Inc., 1948. (*b*) Brues, A. M., and Reitz, L.: Effects of External and Internal Radiation on Cell Division, Ann. New York Acad. Sc. **51**:1497, 1951. (*c*) Burstone, M. S.: Studies on the Alterations of Development of the Teeth and Adjacent Structures Following Local Administration of Chromic Phosphate Containing P^{32}, J. Am. Dent. A. **42**:418, 1951.

2. The radioactive chromic phosphate was obtained from Abbott Laboratories, North Chicago, Ill., on allocation from the Atomic Energy Commission.

3. Gersh, I.: Application in Pathology of the Method of Fixation by Freezing and Drying of Tissues, Bull. Internat. A. M. Mus. **28**:179, 1948.

paraffin (melting point 62 C.) and sectioned serially at 6 μ. Sections were deparaffinized in petroleum ether, dry mounted on albuminized slides, and studied by means of certain histochemical methods as well as routine staining procedures.

For orientation and morphological study sections were stained with hematoxylin and eosin.

A group of experimental and control sections was denatured overnight in absolute ethyl alcohol and then stained by a modification of the Feulgen method which is considered to be specific for desoxyribonucleic acid (DNA).[4]

Other irradiated and normal sections were incubated in a pH 9 phosphate buffer M/15 for one hour at 37 C., denatured in alcohol overnight, and then stained with the Feulgen technique.

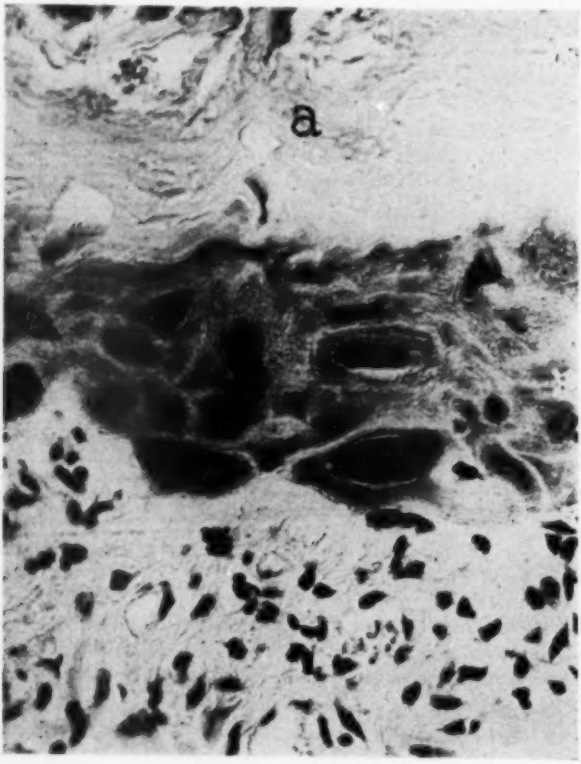

Fig. 1.—Dorsum of tongue stained with hematoxylin and eosin seven days after irradiation. Note the enlarged atypical nuclei and the heavily keratinized surface (*a*); × 650.

This was done so that possible differences in water solubility of nuclear material of normal and irradiated nuclei might be observed.

Still other sections were treated with the enzyme desoxyribonuclease[5] for one hour at 37 C., denatured in alcohol overnight, and stained by the Feulgen method. The desoxyribonuclease was used in a concentration of 1 mg. per milliliter in M/10 barbital (veronal®) buffer at pH 6.8

4. Stowell, R. E.: Feulgen Reaction for Thymonucleic Acid, Stain Technol. **20**:45, 1945. Stowell, R. E.: The Secificity of the Feulgen Reaction for Thymonucleic Acid, ibid. **21**:137, 1946.

5. Worthington Biochemical Sales Company, Freehold, N. J.

with 0.003 M $MgSO_4$. This is a modification of the buffers used by McCarty [6] and Gilbert, Overend, and Webb.[7] Control slides were incubated in the buffer alone.

Sections of both normal and irradiated tissues were stained by a modification of the Unna-Pappenheim, methyl green-pyronine method,[8] which is believed to give an indication of the state of aggregation of nucleic acids.[9] The modification described by Cowdry [10] was used with the following exceptions: The methyl green was extracted repeatedly with chloroform in order to remove the crystal violet which is usually present. The dye substances were dissolved in a pH 4.1 acetate buffer which has been shown to result in a greater stability of methyl green to light.[9]

Still another group of sections was treated with desoxyribonuclease for 30 and 60 minutes at 37 C. and then stained with methyl green-pyronine.

Fig. 2.—Dorsum of normal tongue stained with hematoxylin and eosin; × 650.

6. McCarty, M.: Purification and Properties of Desoxyribonuclease Isolated from Beef Pancreas, J. Gen. Physiol. **29**:123, 1946.

7. Gilbert, L.; Overend, W. G., and Webb, M: The Specificity of Ribonuclease and of Desoxyribonuclease, Exper. Cell Res. **2**:138, 1951.

8. National Aniline Division, Allied Chemical & Dye Corporation, Colour Index 685 (methyl green) and 471 (pyronine B).

9. Kurnick, N. B.: Methyl Green-Pyronin: I. Basis of Selective Staining of Nucleic Acids, J. Gen. Physiol. **33**:243, 1950.

10. Cowdry, E. V.: Laboratory Techniques in Biology and Medicine, Baltimore, Williams & Wilkins Company, 1948.

Metachromasia of irradiated and normal nuclei was observed by studying wet preparations stained with toluidine blue (1:1,000).[11] Studies have indicated that the interaction of nucleic acids with certain dyes to produce a metachromatic staining reaction may be related to the state of aggregation of the nucleic acid.[12]

OBSERVATIONS

Hematoxylin and Eosin.—The irradiated epithelium of the dorsum of the tongue was thin as compared with that of normal controls. Normal outline of the filiform papillae was largely obliterated, but nevertheless the surface was more heavily

Fig. 3.—Higher magnification of irradiated epithelium, showing details of atypical hyperchromatic nuclei. Stained with hematoxylin and eosin; × 1,100.

keratinized. The irradiated epithelium did not exhibit a distinct basal-cell layer and consisted of two to four layers of cells characterized by enlarged hyperchromatic nuclei (Figs. 1, 2, 3, and 4).

11. Bensley, R. R., and Bensley, S. H.: Handbook of Histological and Cytological Technique, Chicago, University of Chicago Press, 1938.

12. Michaelis, L.: The Nature of the Interaction of Nucleic Acids and Nuclei with Basic Dyestuffs, in Cold Spring Harbor Symposia on Quantative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1947, Vol. 12, p. 131.

Feulgen Method.—With the Feulgen method the clumps of chromatin in the irradiated nuclei stained heavily. The stainability was greater than that of the superficial cells of the normal epithelium. It should be noted that nuclei of normal basal cells are intensely Feulgen-positive, but the distribution of stainable material is much more homogenous than that observed in the irradiated nuclei (Figs. 5 and 6).

Both irradiated and normal sections treated with the pH 9 buffer for one hour revealed some decrease in Feulgen stainability as compared with untreated controls. Staining differences between normal and irradiated sections were not apparent.

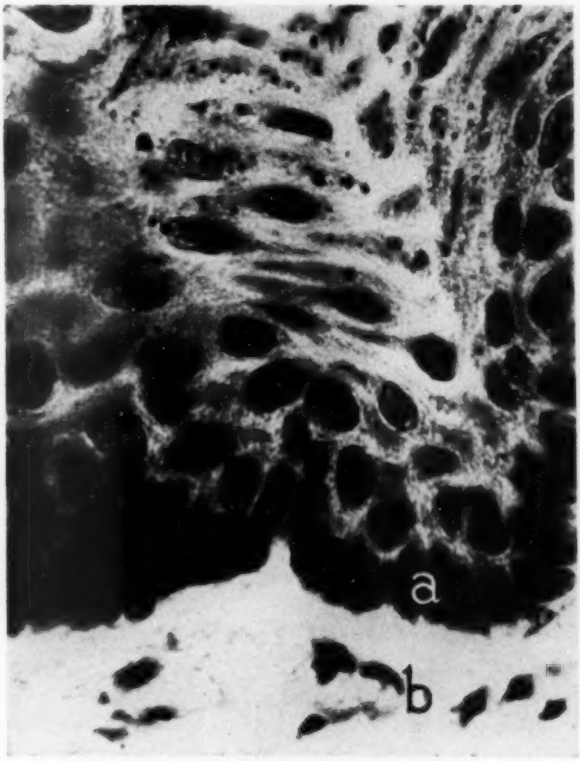

Fig. 4.—Higher magnification of normal epithelium, × 1,100; (*a*) basal cell layer; (*b*) lamina propria.

Treatment with desoxyribonuclease removed Feulgen-positive material from both irradiated and normal nuclei. However, the irradiated nuclei exhibited the greatest decrease in stainability. The irradiated nuclei contained very pale-staining blurred clumps of chromatin, while the normal nuclei revealed only a moderate decrease in stainability. Sections incubated in the barbital buffer minus the enzyme revealed a moderate decrease in stainability as compared with untreated slides.

Methyl Green-Pyronine.—The masses of chromatin in the irradiated nuclei stained with methyl green. Indistinct aggregates of reddish-staining material were also seen but were difficult to interpret. The chromatin of normal nuclei also stained green but more homogenously. Some normal nuclei contained a distinct pyronine-staining body, presumably the nucleolus. It is interesting to note that the cytoplasm of the irradiated cells stained more intensely with pyronine than did that of normal cells.

Treatment with desoxyribonuclease prior to staining resulted in a greatly diminished methyl green staining of the irradiated nuclei. Normal nuclei also stained less

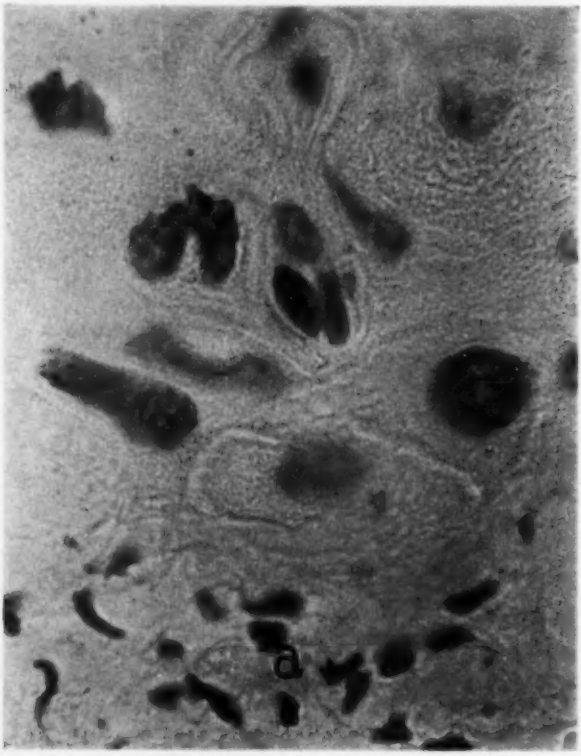

Fig. 5.—Irradiated epithelial cells stained by the Feulgen method, showing aggregates of desoxyribonucleic acid; × 1,100.

intensely after enzyme treatment, but the decrease was not as great as that observed in the irradiated material.

Metachromasia with Toluidine Blue.—The irradiated nuclei contained aggregates which were strikingly metachromatic. Nuclei of normal basal cells were also metachromatic, but to a less marked degree. The more superficial normal epithelial layers showed a progressive decrease in nuclear metachromasia.

COMMENT

Continuous irradiation of moderate intensity from a locally injected suspension containing radioactive phosphorus resulted in striking nuclear changes in the tongue. Preliminary work has indicated that irradiation of this intensity will not result in extensive necrosis of tissue and that the epithelium of the tongue appears relatively normal within a month.

Greatly enlarged nuclei of the irradiated epithelium exhibited clumps of chromatin which stained with the Feulgen procedure. Treatment of tissue sections with desoxyribonuclease resulted in a diminished staining of nuclear material with the

Fig. 6.—Feulgen-stained normal epithelium. The basal cell nuclei (*a*) stain more intensely than the more superficially located nuclei; (*b*) lamina propria; × 1,100.

Feulgen method or with methyl green. As previously indicated, the methyl green stain has been related to the state of organization of nucleic acids. In vitro studies of Kurnick [8] have indicated that depolymerized solutions of desoxyribonucleic acid, as well as nuclei of cells subjected to depolymerizing agents, exhibit a diminished staining reaction with methyl green. The present findings, which indicate an increased susceptibility to enzymatic breakdown of nuclear chromatin following irradiation, suggest that the desoxyribonucleic acid is in a less-aggregated state

than normal. Furthermore, the change in nuclear metachromasia observed in the irradiated tissues may also be related to an altered state of aggregation.

Although a decrease in Feulgen-staining nuclear material (desoxyribonucleic acid) has been reported by Stowell [13] and Warren, Holt, and Sommers,[14] their findings are not necessarily at variance with those herein described. Stowell studied tumor transplants which received multiple exposures of x-ray irradiation comparable to heavy therapeutic doses. It is possible that heavily irradiated tissues exhibit a decrease in Feulgen-positive nuclear material, since there may be definite necrosis of tissue. Differences may also be related to the effects of single heavy doses of ionizing irradiation as contrasted with continuous irradiation of less intensity from P^{32}. It should be mentioned that in Stowell's work tissues were fixed in aqueous fixatives, which may have resulted in the loss of water-soluble nuclear components.

Warren, Holt, and Sommers [14] have found a decrease in Feulgen stainability of various tissues of mice irradiated systemically with P^{32}. In their experiment the frozen-dried tissues were studied during a 24-hour postirradiation period. This stage is not entirely comparable to the one-week postirradiation stage of the tongue observed in the present study.

Mitchell [15] used ultraviolet photomicrography in a study of biopsy specimens of irradiated lesions in human beings. He observed some changes in nuclear ultraviolet absorption. However, a striking increase in the absorption of the cytoplasm was a more consistent finding. Mitchell interpreted this result as indicating an increased nucleic acid content of the cytoplasm. He suggested that irradiation results in an inhibition of synthesis of desoxyribonucleic acid and an accumulation of ribonucleotides in the cytoplasm. The possibility of increased cytoplasmic nucleic acid constituents is further augmented by the observation of the present work that the cytoplasm of irradiated cells stains more intensely with pyronine than does that of normal cells.

CONCLUSIONS

Continuous local irradiation of the epithelium of the tongue with dosages producing a minimal tissue necrosis results in the formation of large atypical nuclei containing masses of chromatin which stain with the Feulgen method and with methyl green.

Treatment of irradiated and normal sections with desoxyribonuclease prior to staining results in a marked diminution in stainability of irradiated nuclei as contrasted with normal nuclei.

It is postulated that continuous irradiation results in the formation of nucleic acid complexes in a less-aggregated state than normal.

Department of Anatomy, University of Chicago.

13. Stowell, R. E.: The Effects of Roentgen Radiation on the Thymonucleic Acid Content of Transplantable Mammary Carcinomas, Cancer Res. **5**:169, 1945.

14. Warren, S.; Holt, M. W., and Sommers, S. C.: Some Early Nuclear Effects of Ionizing Radiation, Proc. Soc. Exper. Biol. & Med. **77**:288, 1951.

15. Mitchell, J. S.: Disturbance of Nucleic Acid Metabolism Produced by Therapeutic Doses of X and Gamma Radiations: Methods of Investigation, Brit. J. Exper. Path. **23**:285, 1942.

DIAGNOSTIC SIGNIFICANCE OF FATTY CYSTS IN CIRRHOSIS

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IN SECTIONS of cirrhotic livers prepared both from choline-deficient rats and from cases of alcoholism in man encountered at autopsy, I have previously reported the almost-constant occurrence of pathologic fatty cysts.[1] These structures have now been demonstrated by three-dimensional reconstructions based on serial sections of cirrhotic livers of man.[2] In experimental animals, it has been possible to trace the stages in development of cysts. Whenever the amount of fat within parenchymal cells becomes sufficiently great, the cells rupture and form cysts as the culmination of the following sequence of events. With progressive enlargement of the fat globules within neighboring cells, the adjacent segments of their limiting membranes caught between the globules become compressed and are eventually fused into a single septum. Although by its formation the septum conjoins the parent cells, it prevents their contained fat globules from coalescing. If the globules continue to enlarge, they eventually so stretch the interposed septum that it is torn. As there is nothing then to separate the fat globules of the torn cells, they coalesce. The pool of fat which results is enclosed, however, by crescentic remnants of the cytoplasm of the parent group of cells, which are still joined together. The fat, now extracellular, is in fact contained within a simple, epithelial cyst (Figs. 1 and 2), which has developed in this manner from fatty liver cells. Cysts, once formed, may continue to enlarge and in turn may fuse, as did the individual cells, with other adjacent cysts or with single, fat-laden cells. Evidence has been presented [3] which demonstrates that when rupture of large cysts (100 μ in diameter in paraffin sections) occurs, bile canaliculi or blood sinusoids may be torn, with the result that fat frequently escapes from the cysts into biliary or vascular channels. Parenchymal and stromal remnants of cysts from which the fat has drained atrophy and become

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1. Hartroft, W. S.: Accumulation of Fat in Liver Cells and in Lipodiastaemata Preceding Experimental Dietary Cirrhosis, Anat. Rec. **106**:61-87, 1950.

2. Elias, H.: Stereograms on Cirrhosis and Fatty Liver, Decatur, Ill., Flint, Eaton & Company, 1951.

3. Hartroft, W. S., and Ridout, J. H.: Pathogenesis of the Cirrhosis Produced by Choline Deficiency: Escape of Lipid from Fatty Hepatic Cysts into the Biliary and Vascular Systems, Am. J. Path. **27**:951-990, 1951.

compressed by the surrounding tissue into "fibrous" trabeculae. These events explain the cirrhogenous nature of fatty cysts and why a fatty liver may become fibrotic.

In later experiments [4] it was found that restoration of adequate amounts of lipotropic factors to the diet of choline-deficient rats in stages of precirrhosis or cirrhosis was followed within a few days by removal of virtually all intracellular fat from the liver. But extracellular lipid contained within fatty cysts might remain for weeks or, in a few instances, months before it was eventually resorbed by the cells in the walls of cysts. When by this means the fat had regained an intracellular position, it rapidly disappeared under the influence of continued choline therapy.

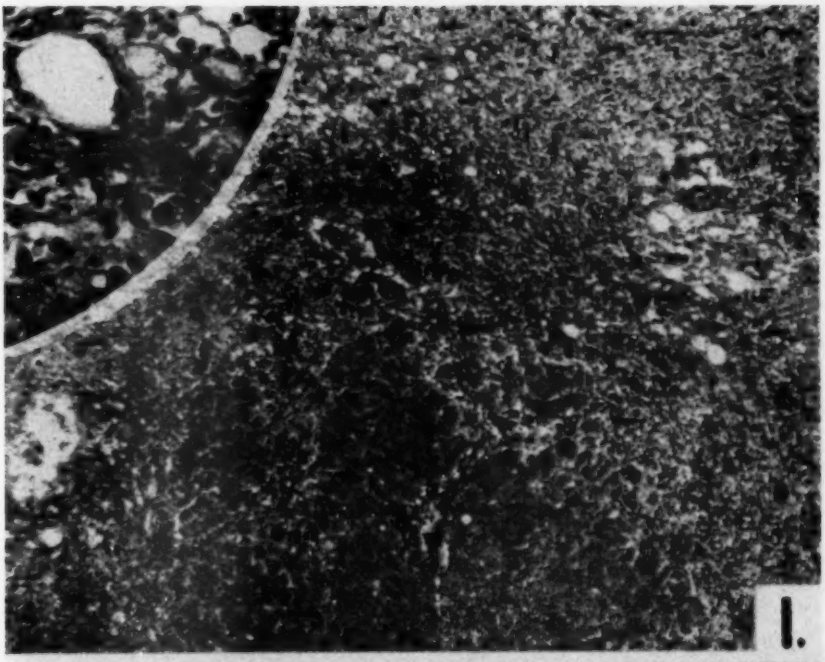
The presence of fatty cysts in any liver, by reason of their pathogenesis, is pathognomonic evidence that at some earlier period pathologic accumulations of fat had distended cells to the point of rupture in those regions of the liver where cysts are found. If in the same areas fibrosis is also present, the nearby cysts constitute presumptive evidence that the fibrosis is the sequel to the formation, rupture, and eventual atrophy of other cysts. Because fatty cysts may remain in livers for long periods after all other stigmata of acute or chronic episodes of extensive fatty metamorphosis have vanished, they may provide the only morphological evidence available to the pathologist that the liver had once been fatty. If the sequelae (fibrosis and cirrhosis) of extensive and prolonged accumulations of liver fat develop, and if the factors initially responsible for the fatty liver become inoperative, only small amounts of extracellular fat within residual cysts may be present at autopsy to indicate the nature of the cirrhosis, for all intracellular fat may have disappeared long before this.

Human beings with cirrhosis sometimes come to autopsy after a considerable period of regression, and in the absence of an adequate history it may be difficult to determine the pathogenesis. It is in such cases that fatty cysts, which are known to survive relatively long periods of regression in experimental cirrhosis, may still be present at necropsy and be of considerable diagnostic significance. Evidence that this view is justified has been obtained from a survey of sections of livers from 95 consecutive, unselected cases of cirrhosis encountered at autopsy. The results, reported in this paper, indicate the degree of diagnostic significance that can be attached to the presence or absence of fatty cysts in cases of cirrhosis of doubtful or unknown etiology.

MATERIAL AND METHODS

The material of the survey consisted of microsections of livers from 95 consecutive cases of cirrhosis of all types encountered at autopsy. These cases were entirely unselected with regard to factors such as age, sex, type of cirrhosis, and its degree or duration. The diagnosis of cirrhosis had been evident in most cases on gross examination, but in others had been based on the presence of lesions which were not detected until the liver was examined microscopically. All sections were systematically searched for the presence of fatty cysts of a special type to be described hereafter. To ensure objective observation while conducting the survey, the investigator did not have access to the case histories or the protocols of the autopsies until the survey was completed. The sections, prepared from blocks of liver fixed in 10% formalin solution and embedded in paraffin, were stained with hematoxylin and eosin. Samples from both right and left lobes were available in nearly all instances, and frequently sections from three to five blocks of tissue were examined for a single liver. In a few instances the results of the examination of

4. Hartroft, W. S., and Sellers, E. A.: The Dissolution of Fatty Cysts in Precirrhotic and Cirrhotic Livers of Choline-Deficient Rats Treated with Lipotropic Factors, Am. J. Path. **28**:387-399, 1952.



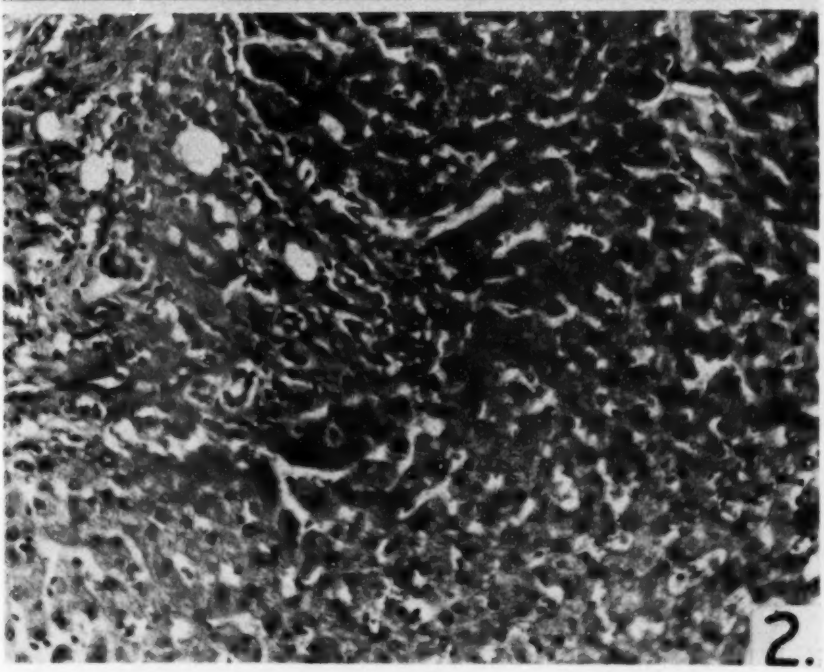
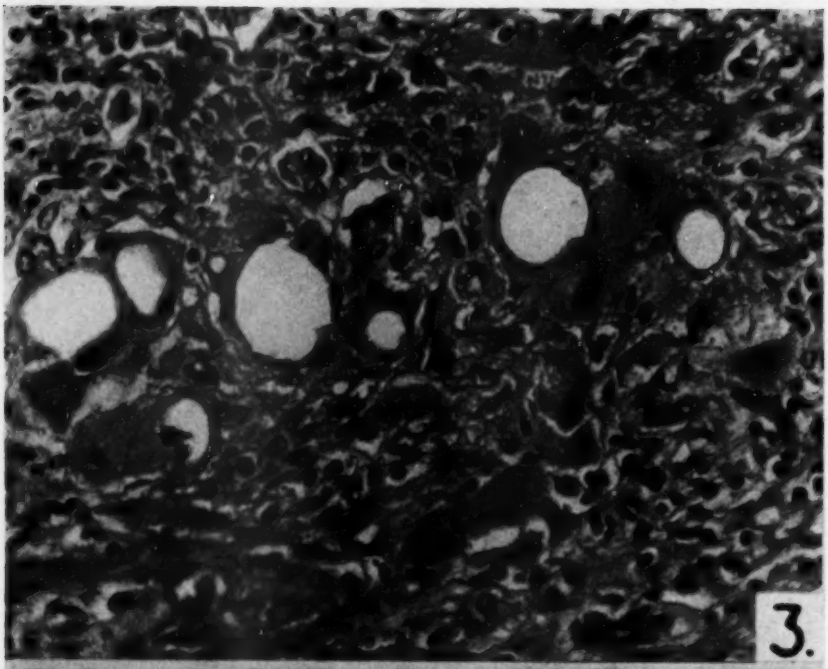


Fig. 1.—Liver of a rat fed a choline-deficient diet for eight months and treated thereafter with lipotropic factors for six weeks. The liver tissue (lower half of field) is almost completely free of fatty vacuolation. Persistent cysts (one of which is shown under higher magnification in the inset, upper left) can be seen scattered throughout the fibrous tissue in the upper right portion. Hematoxylin and eosin; × 100 and × 850.

Fig. 2.—Liver of a 38-year-old man with alcoholic cirrhosis. Despite the absence of fatty vacuolation in the parenchyma at the right, persistent intratrabecular cysts may be seen at the left. Hematoxylin and eosin; × 200.



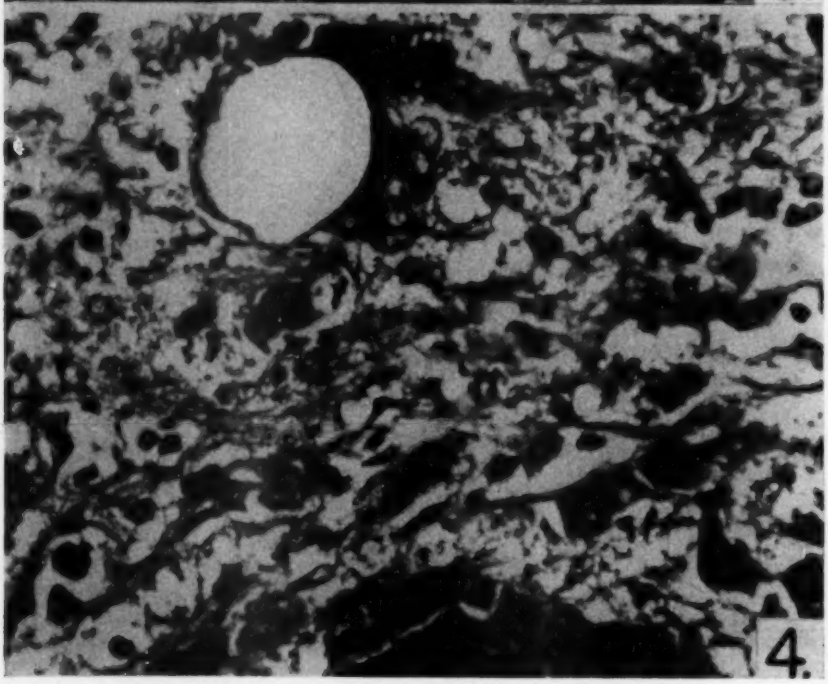


Fig. 3.—Liver of a rat with an experimental history similar to that illustrated in Figure 1. The parenchymal cells in the walls of some of the intratrabecular cysts are still recognizable as such. Hematoxylin and eosin; × 850.

Fig. 4.—Liver of 34-year-old woman with alcoholic cirrhosis. A single, large intratrabecular cyst is shown in the upper portion. Hematoxylin and eosin; × 850.

paraffin sections were confirmed by subsequent inspection of frozen sections which had been stained to demonstrate fat (Sudan IV or Oil Red O). Examination of large numbers of sections from a single liver was deliberately avoided in this investigation in order that the method would conform to that generally employed by pathologists in the ordinary microscopic examination of the liver at autopsy.

RESULTS AND CONCLUSIONS

Before reporting the results of the survey, a brief description will be given of the only type of fatty cysts considered to afford reliable diagnostic aid in interpreting the nature of cirrhotic lesions at autopsy.

Intratrabecular Fatty Cysts.—During relatively brief episodes of abnormal accumulation of fat in the liver, cysts may form which do not progress to the stages of rupture and fibrotic replacement (and thus initiate cirrhosis). Such incidentally formed cysts could appear in livers in which cirrhosis of nonfatty origin either was previously present or developed subsequently. If these cysts, which lack any etiological relationship to the coexistent cirrhotic lesions, were still present at autopsy,

Summary of Data on Cirrhotic Livers Examined at Autopsy

Conditions Associated with Cirrhosis	Cases of Intratrabecular Fatty Cysts	Total Cases Examined
Alcoholism	16	20
Diabetes	9	9
Obesity of pathological degree not complicated by other conditions	9	9
Nutritional deficiencies (starvation and "food fads")	4	4
Pernicious anemia	3	3
Banti's disease	0	3
Postnecrotic scarring	0	4
Biliary tract disease	0	5
Secondary tumors	0	1
Chronic passive congestion	0	6
Unclassified	11	31
Totals	52	95

they could mislead the observer as to the true nature of the cirrhosis were he tempted to attach any diagnostic significance to their presence. This type of error was avoided, or at least minimized, in this study by the investigator's taking cognizance of only those fatty cysts which were found within fibrous trabeculae (Figs. 3 and 4). In sections of cirrhotic livers of choline-deficient rats, cysts were frequently still recognizable even after they had lost most of their contents and were undergoing atrophy and incorporation into trabeculae (Fig. 3). These intratrabecular cysts were found most frequently near the borders of the bands of scar tissue. It would appear unlikely that fatty cysts which had formed independently of cirrhotic lesions of nonfatty origin would even rarely become so intimately associated with the latter as to become embedded in the trabeculae. On this predication, the presence or absence of only the intratrabecular type of fatty cysts was recorded in the course of the survey.

The results of the survey are summarized in the table.

The incidence of intratrabecular cysts could be correlated neither with age, weight, or sex of the patient nor with the severity or duration of symptoms and signs associated with hepatic dysfunction. This observation is in agreement with the experience with experimental animals, in which it is known that the formation of

cysts is independent of these factors. The cysts are present at all stages in either the development or the early regression of cirrhosis produced experimentally by inducing excessive accumulation of liver fat, as exemplified by choline deficiency. Conversely, the cysts have never been found in experimental types of cirrhosis which have not been preceded by severe fatty change, such as that following administration of hepatotoxins (carbon tetrachloride and so on).

With only four exceptions intratrabecular cysts were a constant finding in all those cases of cirrhosis in which the available history indicated that the cirrhosis was probably the sequel of excessive fat storage (alcoholism, 16 of 20 cases; diabetes, 9; obesity, 9; nutritional deficiencies, 4; pernicious anemia, 3). Without exception, cysts were not found in cases in which the diagnosis indicated that the cirrhotic lesions were unlikely to have been the sequel to a fatty liver (Banti's disease, three; postnecrotic scarring, four; biliary tract disease, five; secondary tumor, one; chronic passive congestion, six).

In many of the liver sections in which intratrabecular fatty cysts were present, there was little, if any, of the familiar intracellular type of parenchymal fatty change which would have indicated the fatty nature of the cirrhosis. These sections resembled those of livers of rats in the experiment already cited,[4] in which animals were treated with lipotropic factors for several weeks after fatty cysts and, in some cases, early stages of cirrhosis had been produced by the feeding of low-choline diets. Intracellular fat was completely absent in most of these rats' livers, but fatty cysts had remained as residual stigmata. The four exceptions to the rule that cysts were present in all cases of cirrhosis in which the history indicated that there had probably been preceding stages of excessive fat storage were cases of alcoholism. After completion of the survey, it was found that the patients had not had access to alcohol for periods ranging from six months to two years. (Two were confined to the hospital as a result of serious fractures, and two were treated in institutions by dietary measures and administration of lipotropic preparations.) We suspect that cysts might have been found in the livers of these patients also, had their period of treatment immediately preceding death been less prolonged.

Nontrabecular types of fatty cysts were sometimes encountered in the parenchyma of the liver when the clinical diagnosis had indicated that the cirrhotic lesions were not the sequel to damaging degrees of fat accumulation. This observation emphasizes that fatty cysts of only the intratrabecular type should be considered of diagnostic significance. These, when present, strongly suggest that the cirrhosis was the result of a previous period of excessive fat storage, even though the only abnormal lipid present at the time of autopsy consists of that contained within a few persistent intratrabecular cysts. Best and associates[5] have recently pointed out that there are at least four general causes of fatty liver (dietary abnormalities, hormonal imbalances, oxygen lack, and toxic or infective processes) and that there is no single treatment for all. This statement might be extended to the condition of cirrhosis, which may be the pathologic sequel to fatty liver. Therefore, although intratrabecular fatty cysts, when present, may indicate the group to which a case of cirrhosis may belong, they do not offer more than a general guide to the nature of the lesions.

5. Best, C. H.; Hartroft, W. S., and Sellers, E. A.: The Protection of the Liver by Dietary Factors, Gastroenterology **20**:375-384, 1952.

Absence of intratrabecular cysts does not appear to be as helpful in establishing the nature of cirrhotic lesions as does their presence, because it would seem that they may disappear in proven cases of fatty cirrhosis that have undergone sufficiently long periods of regression as a result of treatment or other factors. If it were known that at the time of death the cirrhosis had not been in a regressive phase, the diagnostic significance of the absence of cysts would be considerably enhanced. Under such circumstances the pathologist would probably be justified in assuming that the cirrhosis had not been produced as a sequel to some disturbance of fat metabolism in the liver and that the cause lay elsewhere.

I believe that the results of this survey indicate that the fibrotic lesions in the 11 cases of unclassified cirrhosis in which intratrabecular cysts were encountered had been produced most likely by earlier episodes of excessive accumulation of fat. Although there was little intracellular fat present in the livers at the time of death, the persistence of cysts within fibrous trabeculae strongly suggests that they are examples of fatty cirrhosis which had undergone regression.

SUMMARY

Ninety-five unselected cases of cirrhosis encountered at autopsy were surveyed microscopically for the presence or absence of fatty cysts within the fibrous trabeculae.

Intratrabecular cysts were found in 52 cases. In all but 11 of these, in which the histories were inadequate, it was established that the cirrhosis was associated with alcoholism (16 cases), diabetes (9 cases), obesity (9 cases), a nutritional deficiency (4 cases), or pernicious anemia (3 cases). Fatty cysts could not be demonstrated in four additional cases of alcoholic cirrhosis, but in these the patients had been treated in institutions for periods ranging from six months to two years prior to the time of their death.

In 43 of the 95 cases surveyed, intratrabecular fatty cysts could not be demonstrated. This observation applies to all those cases in which the histories indicated that the cirrhosis had not been preceded by excessive accumulation of liver fat, including the instances of Banti's disease (three cases), postnecrotic scarring (four cases), biliary tract disease (five cases), secondary tumor (one case), and chronic passive congestion (six cases).

Of the cases in the series, 31 were of unknown etiology, and of these, intratrabecular fatty cysts were present in 11. On the basis of the results of the survey of known types of cirrhosis, it is suggested that the presence of intratrabecular fatty cysts in these 11 cases probably indicates that they should be regarded as instances of fatty cirrhosis, even though little intracellular fat was present at the time of death.

ELASTIN CONTENT OF THE ALBUMINOID FRACTION OF HUMAN AORTAE

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RECENTLY we reported the presence of a carbohydrate fraction in the human aortic albuminoid and demonstrated that this carbohydrate fraction showed no significant quantitative changes with age, sex, or pathology.[1] In further work with the albuminoid fraction it was decided to study the percentage of elastin, or the collagen-elastin ratio. Troitzka-Andreva [2] reported a gradual increase of collagenous substance with increasing age. Lansing, Alex, and Rosenthal,[3] Hass,[4] and Myers and Lang [5] studied the percentage of elastin in aortae, and Smith, Seitner, and Wang [6] reported increases in elastic tissue and collagenous fibers of the media with aging.

MATERIAL AND METHODS

Fresh thoracic aortae obtained at autopsy were examined carefully to note their condition as to atheromatous plaques and degree of calcification so that these data could be checked later with autopsy findings. The albuminoids were prepared by methods previously described,[1] except that sodium chloride and dibasic sodium phosphate soakings were substituted for the calcium hydroxide treatments. The refrigerated tissues, subsequent to acetone and alcohol extractions, were soaked in three changes of sodium chloride for a minimum of 24 hours during each extraction period. They were then treated with M/15 dibasic sodium phosphate for two soaking periods of at least 24 hours in the refrigerator. After five 24-hour washings in distilled water, the samples were dehydrated with several changes of absolute alcohol, dried in an oven at approximately 70 C., and desiccated for at least 24 hours. Each sample of albuminoid after being weighed accurately was hydrolyzed in approximately M/10 sodium hydroxide one or more times for at least one hour each time. The hydrolysate was removed with a pipette and the hydrolysis was repeated with fresh sodium hydroxide until the hydrolysate was colorless. This part of the procedure took from a little less than 4 hours for infant aortae to 18 hours for highly sclerotic aortae. It is stressed because care was taken to remove completely the collagen portion from the elastin part of the albuminoid fraction. After the residue was washed several times with distilled water to remove the sodium hydroxide and dehydrated several times with absolute alcohol, it was dried in a 70 C. oven, desiccated, and weighed. The elastin thus prepared on an empirical chemical basis contained minerals, including calcium salts, which would not have been dissolved.

From the Department of Anatomy, State University of New York College of Medicine at New York City, Brooklyn.

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RESULTS

The 43 aortae used in this study were from persons who ranged in age from newborn to 89 years. The mean percentage of elastin in the albuminoid fraction did not significantly change with age (Table). In addition, the correlation coefficient showed no significant relationship between age and percentage of elastin ($r = +0.26$).

It was found also that there were no sex differences in the mean percentage of elastin in the albuminoid fraction. The accompanying table shows that the mean percentages of elastin for the males and females were by coincidence the same, namely 34.8, and this was the same as the mean percentage for all ages.

The aortae were divided on the basis of gross observations into five groups: 0, normal; 1+, a few atheromatous plaques; 2+, moderate atheromatous plaques;

Elastin Content of the Albuminoid Fraction of Human Aortae

Age, Yr.	No. of Samples	Mean % of Elastin in Albuminoid Fraction	Standard Deviation	t	Probability
0-39	10	32.1	5.5	....	....
40-69	16	34.4	9.3	0.69	0.50
70-89	16	36.5	10.1	1.25	0.25
All ages	43	34.8	8.9	....	....
Sex					
M	23	34.8	8.0	....	....
F	20	34.8	10.0	....	....
Condition *					
(0)	11	30.8	6.8	....	....
(+)	8	32.8	9.1	0.6	>0.50
(++)	2	31.4	11.1	0.01	>0.50
(+++)	16	36.5	6.8	2.1	0.04
(++++)	6	41.7	13.3	2.3	0.04
All pathological aortae	32	36.2	9.1	1.8	>0.05

* 0, normal; +, a few atheromatous plaques; ++, moderate atheromatous plaques; +++, atheromatous plaques and a few calcified plaques; ++++, many plaques, atheromatous and calcified.

3+, atheromatous plaques and a few calcified plaques, and 4+, many plaques, atheromatous and calcified. This classification agreed essentially with the autopsy data. In comparing the 32 pathological with the 11 normal aortae it was found that the mean percentage of elastin for the pathological group was 36.2 and for the normal group 30.8. This is not a statistically significant difference, although the t of 1.8 and probability of greater than 0.05 indicated that it was nearing significance. The 3+ and 4+ groups, with 36.5 and 41.7 for the mean percentages of elastin, exhibited increases as compared with the 30.8 value for the normal group and the 32.8 and 31.4 percentages of elastin for the 1+ and 2+ groups. These increases in percentage of elastin in the 3+ and 4+ groups, which showed calcified plaques, were statistically significant. This variation was not due to chance alone since the chi square test showed that the probability value was less than 0.05.

The results of this investigation thus revealed that the percentage of elastin in the albuminoid fraction of the human aorta did not change significantly with age, sex, or atheromatous condition as long as there was no calcification. In aortae in which the plaques were calcified as well as atheromatous there was a statistically significant increase in mean percentage of elastin.

COMMENT

It is difficult to compare results for percentage of elastin, since in most experiments different methods were used on different portions of the aortic wall. In addition, although the best available methods have been used, they are all somewhat crude. However, the results of this study were in accord with those of Hass,[4] who analyzed whole aortae and found that the quantity of elastic tissue in each unit volume of average aortic wall remained nearly constant throughout life. The average which he reported for 21 aortae from persons ranging in age from 10 days to 77 years was 37.9%. Lansing, Alex, and Rosenthal [3] reported on 110 cases, the ages ranging from newborn to 103 years, in which they used defatted, desiccated media for study. They reported that during the first two decades the average percentage of elastin content of the media was slightly over 48, while in the third decade and thereafter the value dropped to between 41.4 and 44.1. They reported that it was difficult to decide whether or not the drop was significant. It is of interest to note that the same group of workers [7] reported for the pulmonary artery a mean percentage of elastin in the media of 30.6 in the newborn to 19 year group, with values increasing until the percentage for the 70 to 79 year group was 36.6. Again, the increase with age was small but the authors reported it to be statistically significant.

The results of the present study, demonstrating an absence of age changes in percentage of elastin, are not in accord with those of Myers and Lang,[5] who used 83 aortae from persons 15 to 88 years of age and took both intima and media for their analyses. They reported elastin as being highest in early life, namely 37.4%, and gradually declining to 26.6% in the seventh decade. Although no statistical analysis was reported, the authors thought there was a significant downward trend at the rate of 3% every 15 years.

A comparison of the entire pathological group with the normal group revealed no significant change in the mean percentage of elastin, although there was an increase from 30.8% in the normal to 36.2% in the pathological group and the probability of greater than 0.05 indicated that the value might be nearing significance. As to the percentage of elastin for the 3+ and 4+ groups, the increases have been shown to be significant. There seems to be a likely explanation for the increase as compared with the normal and with the two lesser pathological states. As was mentioned, the method of separation of constituents is such that undissolved minerals, such as calcium salts, remain with the elastin residue. It may well be that the increase in percentage of elastin observed is due to the presence of calcium in these two groups.

SUMMARY AND CONCLUSIONS

1. There was no statistically significant change in mean percentage of elastin in the albuminoid fraction of human aortae with age, sex, or atheromatous condition.

2. The mean percentage of elastin for the 43 aortae, including specimens from both sexes, was 34.8.

3. For the atheromatous group the percentage was 36.2, as compared with 30.8 for the normal group.

4. There was a statistically significant increase in mean percentage of elastin in the pathological aortae which were observed grossly to contain calcium.

Miss Jean Wright provided technical assistance, and Dr. I. M. Murray and Mr. G. Mestler performed the statistical analyses.

7. Lansing, A. I.; Rosenthal, T. B., and Alex, M.: J. Gerontol. **5**:211, 1950.

VASAL COMPONENT IN SYNDROME OF FOIX AND ALAJOUANINE

"Subacute Necrotizing Myelitis"

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AMONG the cases of necrosis of the spinal cord without inflammatory features, which are classified in the literature as instances of "subacute necrotizing myelitis," or the syndrome of Foix and Alajouanine, one group is characterized by the presence of peculiar vascular changes. Foix and Alajouanine [1] described proliferative endomesovasculitis and venous dilatation as outstanding lesions in their two cases. Subsequently, other authors published cases labeled with this diagnosis in which vascular alterations were absent or insignificant. Moersch and Kernohan [2] suggested the term "progressive necrosis of the spinal cord" for this disease, and they thought that the importance of the inconstant vascular lesions may have been overemphasized. Like Foix and Alajouanine, they believed that the disease was toxic, or possibly viral, in origin. Greenfield and Turner [3] summarized the clinical picture of the syndrome: progressive amyotrophic paraplegia, dissociated sensory changes, loss of tendon reflexes and sphincter control, and elevated spinal fluid protein. In their three cases, the parenchymal changes of the cord were secondary to obliterative sclerosis of the small intramedullary and meningeal vessels and associated thickening of the walls of the larger meningeal veins and sometimes of the arteries. Jaffe and Freeman [4] reviewed and tabulated the cases previously reported in the world literature. More recently, the occurrence of associated vascular malformations, such as "spinal varicosis," has been described, especially by Suter-Lochmatter.[5] We are not aware of any recent American publications, except for textbooks, that deal with this subject.

REPORT OF CASES

CASE 1.—A 67-year-old white man, a diabetic, while recovering from the resection of a carcinoma of the maxillary sinus, had a spinal puncture. Immediately after this procedure he complained suddenly of weakness of the legs and was barely able to walk a few steps to bed. Neurological examination revealed diffuse muscular weakness of the lower extremities, severer

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on the left. The Babinski and Kernig signs were absent. The triceps, biceps, abdominal, and cremasteric reflexes were active. The patellar and ankle reflexes were absent. The right thenar muscles were atrophic. (He stated that his hand had been weak for six years and that since the age of 17 his left leg had been weak when he was under emotional stress.) No sensory changes were elicited. He rapidly became incontinent and required a retention catheter continuously. The spinal fluid contained no cells; the protein was 90 mg. per 100 cc., the sugar content was 165 mg. per 100 cc., and the Wassermann reaction was negative. The diabetes was readily controlled, and the urine was found to contain sugar on only two days. He was not hypertensive. After 10 weeks of gradual improvement, he was able to stand and take a few steps, and he was discharged from the hospital. He was readmitted six months later complaining of progression of the paralysis, with painful cramping of the calves and numbness of the legs. He had hyperesthesia of the genital area. The calf muscles were atrophic, and the upper extremities also appeared weak. Only the abdominal and ankle reflexes were absent. The superficial and deep pain and tactile senses appeared normal. Position sense

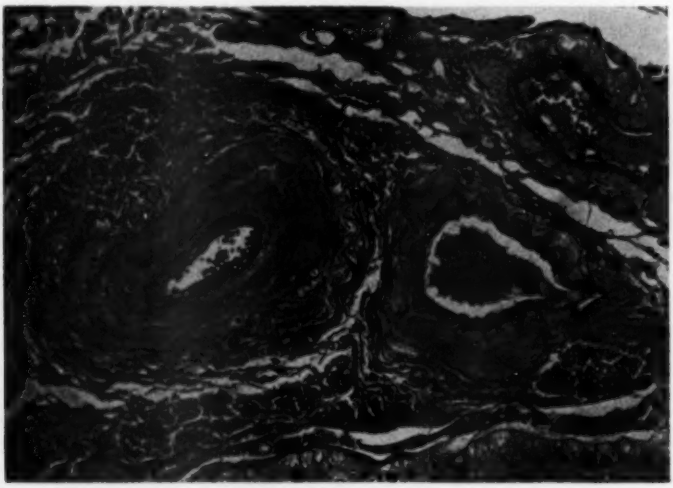

Fig. 1 (Case 1).—Hyaline sclerosis of leptomeningeal veins. Hematoxylin-eosin stain; × 180.

was present in the upper extremities but was absent in the lower. He remained incontinent. A large sacral decubital ulcer developed. He became gradually weaker and died quietly 11 months after onset of the illness.

Autopsy.—Coronary arteriosclerosis and a healed infarct of the interventricular septum were observed. Generalized arterial and arteriolar sclerosis were absent. No other significant visceral lesions were found. The brain was not remarkable. The subdural space of the spinal cord was filled completely with soft translucent gray fibrous tissue from the midthoracic to the sacral level. The cauda equina was embedded in the abundant meningeal connective tissue. Calcified foci were present in the dura mater. The spinal cord itself did not appear abnormal. Spinal varices were not observed.

Microscopic Examination.—Microscopically, the spinal dura showed fibroblastic and hyaline fibrous thickening, with occasional granules of embedded calcium. The vessels, predominantly the small veins, exhibited severe diffuse lamellated fibrosis of the walls, swollen endothelial lining cells, and moderately wide lumens. The leptomeninges showed advanced fibrosis and contained many veins of similar appearance with diffusely hyalinized walls, which were almost devoid of nuclei (Fig. 1).

Slightly wavy concentric collagen fibers, of varying density, comprised the wall and blended with the surrounding dense connective tissue. Vessels of all sizes were involved. In most instances the vessels were patent or moderately dilated. A few collapsed, ectatic, thin-walled veins also were present. Occasional veins, however, were obliterated by loose webby non-nucleated fibrous tissue, which merged with the denser collagen of the wall. Numerous small vessels showed concentric fibrosis, resembling the "onion-skin" vessels of Foix and Alajouanine (Fig. 2). The inner portion of the wall of occasional small vessels exhibited severe obstructive cellular proliferation. The anterior spinal artery showed splitting of the internal elastic membrane and cellular-fibrous hyperplasia of the intima (Fig. 3). The anterior spinal vein also possessed a fibrotic wall, and at some levels the lumen was markedly dilated.

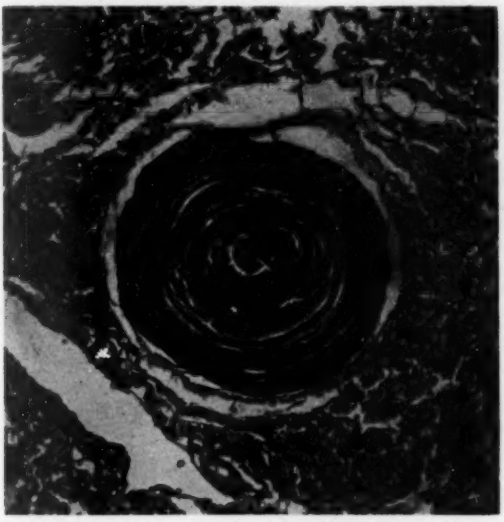

Fig. 2 (Case 1).—Obliterated "onion-skin" dural vessel. Hematoxylin-eosin stain; × 180.

The nerve roots contained a surprisingly large number of thick-walled venules with wide lumens; the walls were homogeneously hyalinized and without nuclei in most places (Fig. 4). Patchy demyelination was present in most of the nerve roots; in some, the majority of nerve fibers were affected; others were normal. Traces of neutral fat were present.

Numerous intraspinal vessels, particularly in the periphery of the cord (Fig. 5), showed extensive thickening and hyalinization of the walls, which blended with the adjacent parenchyma. The endothelial cells were prominent. Many of these vessels had broad lumens. The reticulum stain showed that the homogeneous walls consisted of layered, coarsened, and fused fiber bundles (Fig. 6). The Van Gieson stain demonstrated that the walls were composed of collagen. The deep vessels in the gray matter were also involved, more in one anterior horn than in the other. The anterior horns showed a great diminution in the number of nerve cells. Some cells,

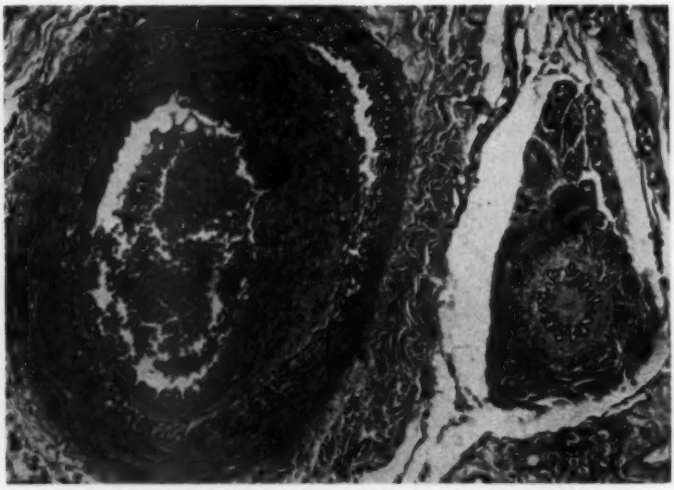

Fig. 3 (Case 1).—Cellular-fibrous intimal proliferation of anterior spinal artery and obliterated arteriole. Hematoxylin-eosin stain; × 180.

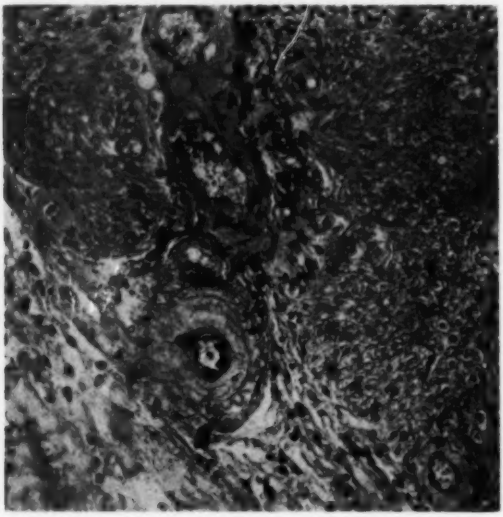

Fig. 4 (Case 1).—Vascular lesions in spinal roots. Hematoxylin-eosin stain; × 180.

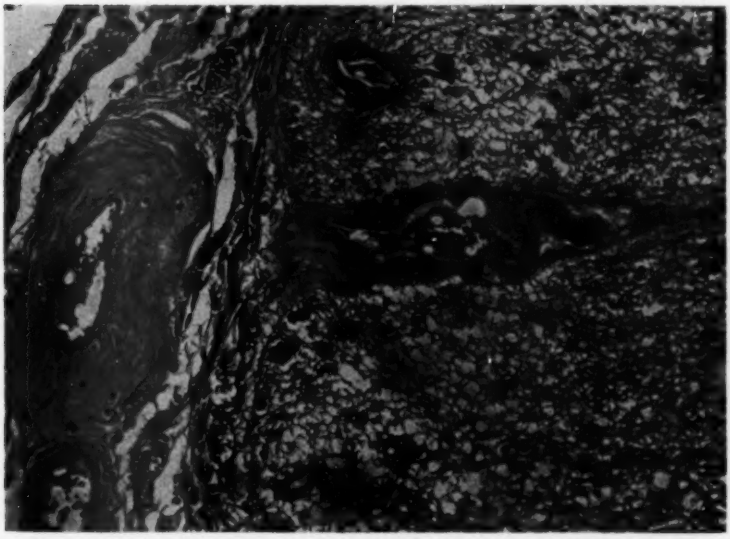

Fig. 5 (Case 1).—Involvement of meningeal and intramedullary veins. Hematoxylin-eosin stain; × 180.

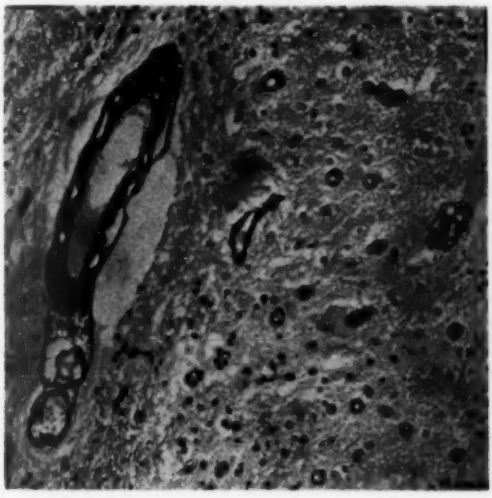

Fig. 6 (Case 1).—Increased vascularity, with thickening of the walls of intramedullary vessels. Reticulum stain; × 180.

particularly in the marginal areas, were well preserved; but others were shrunken and pale, and ghost cells were present. Some cells had distorted nuclei, fragmented nucleoli, and faintly visible cytoplasm. In the areas of neuronal devastation there was pronounced plasmatic gliosis, with occasional clusters of swollen astrocytes. Marginal degeneration of the myelinated fibers, less accentuated around the median fissure, and diffuse, incomplete demyelination of the posterior funiculi were present. Gitter cells were scattered through these areas. The Braunmühl stain revealed swelling, fragmentation, and loss of axis-cylinders in the demyelinated areas. Recent necrosis was not observed.

Case 2.—A woman aged 58 experienced sudden pain in the left arm and shoulder, which, after transient improvement with physical therapy, progressed in a few days to what was stated to be a "modified Brown-Séquard syndrome." Three weeks later she became incontinent of bowel and bladder and had a urinary infection. One month after the onset of the infection quadriplegia developed, with bilateral loss of sensation from the shoulders down. Examination revealed shallow, gasping respiration, pallor, and cyanosis; the neck was stiff and painful on palpation. The knee jerk was hyperactive; the ankle jerk was not elicited; the Babinski reflex was present on both sides. The heart rate was rapid and irregular (120 to 130); the blood pressure was 130/100, and the temperature was 102 F. A roentgenogram of the cervical portion of the spine showed a normal condition. An emergency laminectomy was done the following night, and discoloration and swelling of the cord were found at the level of the fifth cervical segment. Clotted blood escaped from a small incision in the cord. The dura was left open for decompression. After transient improvement, the patient died, less than 24 hours after the operation.

Autopsy.—Postmortem examination was limited to the removal of a small piece of spinal cord from the site of the operation. The specimen consisted of a segment measuring 5 cm. in length from the cervical portion of the cord. The surface of the cord failed to show vascular anomalies. The cord was slightly enlarged; the nerve substance was pushed to the periphery, and the bulk of the tissue consisted of old and recent blood clots.

Microscopic Examination.—Microscopically, the hemorrhagic areas consisted of poorly stained red blood cells, occasional lymphocytes, necrotic particles of nerve tissue, and some scavenger cells containing blood pigment. The hematoma was walled off by a capsule of collagenic connective tissue, which varied in thickness and contained small clusters of hemosiderin cells. In places, delicate collagenic fibers spread from the wall in to the hematoma. The tissue of the spinal cord was extremely compressed; however, some of the large nerve cells of the anterior horns were still present and occasionally showed features of retrograde degeneration. The myelin sheaths were well preserved in part, and the nerve roots showed only slight demyelination. Many areas of vacuolation, or "lacunar degeneration," were noted in the periphery of the cord. Some of the vacuoles contained small homogeneous pink droplets, which appeared brown to brownish-red with the Van Gieson stain. In a few places the droplets blended with the walls of neighboring vessels. Occasional larger patches of homogeneous plasmatic material with similar staining reactions were seen. Scattered small droplets of fat were found, mainly within proliferated microglial cells. No gitter cells were observed. A few very small scars were noted in the white matter.

Many of the small vessels, which were preserved in the periphery of the hematoma, exhibited normal or pyknotic endothelial cells and thickened, homogeneous, or lamellated walls, which lacked distinct demarcation from the parenchyma (Fig. 7 *A*). They stained yellowish-brown to brownish-red with the Van Gieson method. Most of the arterioles and venules visible elsewhere in cross sections of the spinal cord were involved in a similar fashion and had rather wide lumens. Tangles

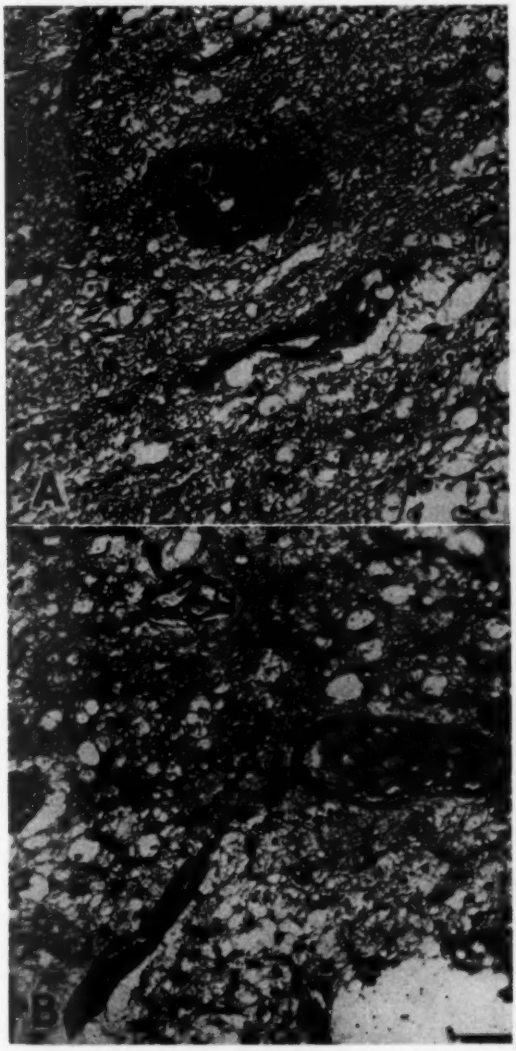

Fig. 7 (Case 2).—*A*, extensive thickening of the walls of small intramedullary vessels. The walls blend with the parenchyma, which shows vacuolar degeneration. Hematoxylin-eosin stain; × 180.

B, thickening and splitting of the intramedullary vascular walls. Perdrau stain; × 180.

and coils of small dilated veins occurred occasionally. The mesenchymal fibers of the vascular walls were prominent with the Perdrau stain (Fig. 7*B*), but elastic fibers were subtotally disintegrated. Generally, the vascularity of the spinal cord appeared increased. Several vessels were surrounded by small numbers of lymphocytes and hemosiderin-laden phagocytes, and in the vicinity some proliferated protoplasmic astrocytes were present. Lymphocytic infiltration was also seen in areas distant from the hemorrhage. Only a few meningeal vessels were contained in the sections, and several of them showed corresponding pathological changes. No evidence of intramedullary neoplasm or indisputable vascular malformation was observed.

COMMENT

In summary, the principal meningeal vascular changes observed by us consisted of thickening and hyalinization of the walls of the vessels with reduction of the number of nuclei. The veins and venules mainly were involved. Frequently, they showed a lamellated, or "onion-skin," appearance. The adventitia blended with the extremely fibrosed meninges. The lumens of most veins were moderately dilated. Complete obliteration by fibrillar collagenic tissue occurred less frequently. A few small vessels exhibited marked cellular proliferation of the intima. The arteries showed similar sclerotic changes, but to a less extent. Sometimes it was difficult to distinguish arterioles from venules. The elastic fibers appeared to have disintegrated, rather than to have proliferated, in the course of the disease. The intramedullary vessels were hypertrophic and apparently numerically increased. The outer walls fused imperceptibly with the surrounding parenchyma, and eosinophilic material flowed out from the lumens. The vascular walls showed homogeneous swelling, fibrosis, and separated or fused coarse, irregular argentophilic fibers. The nerve roots contained a number of enlarged patent hyalinized vessels.

The vascular alterations resembled those described by Foix and Alajouanine [1]; van Gehuchten [6]; Lhermitte, Fribourg-Blanc, and Kyriaco [7]; Minea [8]; Markiewicz [9]; Greenfield and Turner [3]; Giampalmo [10]; Juba [11]; Gagel and Mészaros [12]; Stolze [13]; Scholz and Manuelidis,[14] and Suter-Lochmatter.[5]

Staemmler,[15] in 1939, described alterations in veins that occur with high frequency in old age, and that have certain similarities to the lesions observed in our cases. He saw in the meninges, in the nerve roots, and in the cord hyaline degeneration of veins, with homogeneous swelling of the wall, apparently progressing inwardly from the periphery, and not leading to narrowing of the lumen. Fatty metamorphosis and vascular calcification also were encountered occasionally. Secondary parenchymal changes were not observed. Although some of these features were present in our cases, on the whole the lesions were more numerous and severer

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8. Minea, I.: Bull. Soc. roum. neurol. **13**:27, 1932.
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14. Scholz, W., and Manuelidis, E. E.: Deutsche Ztschr. Nervenh. **165**:56-71, 1951.
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and showed greater variety; furthermore, parenchymal changes were associated with them. Therefore, the lesions appeared to be more than only an accentuation of the normal aging process. Although the first patient was diabetic, the diabetes was a minor feature of the entire clinical course, and the vascular lesions were not particularly like those seen in other organs in diabetes. The changes were also different from those of ordinary arteriolar or arterial sclerosis or phlebosclerosis.

The peculiar blending of broadened vascular walls with the surrounding ground tissue, into which material from the vessels appeared to spread, occasionally with the formation of patches of plasmatic substance, pointed to "dysoria," i. e., a disturbance in the barrier function of the endothelium. This term was coined by Schürmann and MacMahon [16] in their monograph on malignant nephrosclerosis. A certain similarity between the vascular lesions in our cases and those of malignant nephrosclerosis cannot be denied. The significance of "dysoria" in the Foix-Alajouanine syndrome was stressed recently by Scholz and Manuelidis; it was believed to be responsible for the parenchymal necrosis, which was seen in various stages in their case.

Several recent European papers have emphasized the occurrence of the Foix-Alajouanine syndrome in patients who have congenital anomalies of the veins. Stolze's case was characterized by a racemose venous hemangioma located at the base of the medulla, and a plum-sized aneurysm of the internal carotid artery. In the case of Scholz and Manuelidis there were typical vascular changes and, in addition, greatly dilated extramedullary veins that resembled varices. The changes in the vessels could not be considered inflammatory, and the authors believed that the term "endomesovasculitis" should be discarded. Because of the hemangiomatous malformation, they recommended that the diagnostic term "angiodysgenetic necrotizing myelopathy" be used. Suter-Lochmatter believed that the disease is a special form of "spinal varicosis," with superimposed chronic stenosing endophlebitis of the varicose veins. She distinguished between a chronic, "neuralgic-paralytic" form and an acute, "apoplectic" type (hematomyelia) of spinal varicosis. She reviewed 23 cases in the literature, mostly those of Wyburn-Mason,[17] and reported three new ones.

In our cases there was no reason to believe that the altered vessels represented congenital anomalies, and obvious varices were not observed. In the first case, in which a complete autopsy was performed, venous anomalies in other locations, compatible with the "status varicosus" mentioned by Suter-Lochmatter, were not demonstrated. Nevertheless, there appeared to be a general increase in the number and size of both the meningeal and the medullary vessels. In the second case it is open to question whether or not the small vascular coils represented a congenital anomaly. Meningeal fibrosis, without evidence of previous inflammation, was a remarkable feature in the first case, and may have been of more significance than one would assume from the literature.

In a reconciliation of the clinical picture with the anatomic findings, the progressive, exceedingly chronic nature of the process in the first case was apparent. Possibly the weakness in the limbs, which the patient complained of for many years,

16. Schürmann, P., and MacMahon, H. E.: Virchows Arch. path. Anat. **291**:47-218, 1933.

17. Wyburn-Mason, R.: The Vascular Abnormalities and Tumours of the Spinal Cord and Its Membranes, London, Henry Kimpton, 1944.

was due to long-standing small focal lesions of the spinal cord. The parenchymal changes were old and may have been present for many months, or even years, but active progression of the degeneration was also evident at the time of autopsy. In the second case clinical evidence of older lesions was lacking. However, the small scars found in some of the sections indicated mild damage to the spinal cord preceding the fatal hemorrhage. It should be emphasized that traumatic injury, which is usually considered the most frequent cause of hematomyelia, did not play a role in this case.

Although the vascular lesions in both our cases greatly resembled those previously described, the parenchymal alterations presented definite differences from those of the other cases of the Foix-Alajouanine syndrome. The diagnosis of "subacute necrotizing myelitis" was not appropriate on histological grounds. In the first case, the degeneration was patently chronic and without inflammatory features. In the second case, the course was acute, and the massive hemorrhage appeared to be a complication. Foci of vacuolar degeneration were like those observed repeatedly in the subacute form of the disease, however. Hematomyelia, to our knowledge, has been described in only one previous case of this syndrome (Suter-Lochmatter); otherwise, only small and insignificant hemorrhages were mentioned in older papers.

If it is true that vascular disease is responsible for the lesions in the spinal cord, it is not surprising to find any form of parenchymal damage which may result from a circulatory disturbance, as well as the originally described necrotizing changes. Our material demonstrates that both chronic degeneration and rapid hemorrhagic destruction of spinal cord tissue are possible. If "dysoria" is an important factor, the extravasation not only of plasma but also of red blood cells from the acutely diseased vessels, with resultant massive hemorrhage, might be anticipated. Evidence of "dysoria" was found histologically in both cases. Inflammation played a minimal part, and we agree with those who believe that such changes are of secondary or symptomatic character (Scholz and Manuelidis). The parenchymal damage was not limited to either gray or white matter. It involved both in an irregular manner, and therefore various combinations of sensory and motor symptoms might be expected in different cases.

Our cases appeared to support the modern trend to separate the spinal cord diseases which exhibit the peculiar vascular changes of Foix and Alajouanine from those in which necrotizing, inflammatory, or purely degenerative lesions dominate. We believe that the former group alone should be given the name Foix-Alajouanine syndrome. It is not justifiable to call all vaguely related diseases of the spinal cord by this term. This, in our opinion, would be just as wrong as to call any lesion of the spinal roots or peripheral nerves the Guillain-Barré syndrome. Unfortunately, there is a great tendency to do such things, for, as the late F. H. Lewey [18] pointed out, "strange-sounding words, even in our rationalistic time, still cast a magic spell and tend to captivate our phantasy," especially when the pronunciation of such names is difficult.

A definite position cannot be taken concerning the etiology of the vascular lesions. We had no proof in our cases that the lesions were dependent upon vascular malformations or "angiodysgenesis," although the recent literature definitely points in that direction. A certain relationship to senile vascular changes appeared to exist.

18. Lewey, F. H.: J. Pediat. **26**:165-177, 1945.

SUMMARY

Two cases of the syndrome of Foix and Alajouanine ("subacute necrotizing myelitis") are described. In one the condition was chronic; in the other it was acute and terminated with hematomyelia. The essential lesions were found in the vessels, especially veins, of the spinal meninges, cord, and nerve roots. They consisted of hyperplasia and hyalinosis of the walls, with altered permeability, and with associated degeneration of the gray and white matter of the spinal cord. The cause of the disease was not determined. Congenital venous malformations were not observed. The vascular changes resembled somewhat those seen in senility. The vasal component was considered to be of primary importance in the Foix-Alajouanine syndrome.

Books

Diabetic Glomerulosclerosis: The Specific Renal Disease of Diabetes Mellitus. By Harold Rifkin, M.D., lecturer in medicine, Columbia University College of Physicians and Surgeons, adjunct attending physician, Montefiore Hospital for Chronic Diseases; Louis Leiter, M.D., Ph.D., clinical professor of medicine, Columbia University College of Physicians and Surgeons; chief, medical division, Montefiore Hospital for Chronic Diseases, and James Berkman, M.D., instructor in pathology, Columbia University College of Physicians and Surgeons; associate pathologist, Montefiore Hospital for Chronic Diseases. Price, $3.50. Pp. 102, with 10 tables and 13 figures. American Lecture Series. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1952.

This book is divided into seven chapters: (1) The Clinical Syndrome, (2) Pathology, (3) Pathogenesis, (4) Analysis of Clinical Data, (5) Laboratory Aids in Diagnosis, (6) Differential Diagnosis, and (7) Treatment. There is a bibliography listing 81 key references and a useful and adequate index.

The authors have succeeded in bringing together in concise form and integrating the clinical, clinical pathology, and pathologic observations related to the renal changes of diabetes mellitus.

This small volume can be recommended to clinicians, pathologists, and students of medicine as a balanced description and interpretation of its subject.

Atlas of Tumor Pathology: Section V, Fascicle 17, Tumors of the Lower Respiratory Tract. By Averill A. Liebow, M.D., professor of Pathology, Yale University School of Medicine. Price, $1.25. Pp. 190, with 249 illustrations. Armed Forces Institute of Pathology, under the auspices of the Subcommittee on Oncology of the Committee on Pathology of the National Research Council, 2101 Constitution Ave., N. W., Washington 25, D. C., 1952.

This latest fascicle in the "Atlas of Tumor Pathology" deals with neoplasms of the lung, pleura, and diaphragm. As might be expected, the greater part is devoted to epithelial tumors with consideration of papillomas and adenomas of the bronchi, bronchiolar carcinoma (alveolar cell tumor), and bronchogenic carcinoma. The discussion of mesodermal tumors includes some of the rarer neoplasms, which are well illustrated. Metastatic tumors are briefly covered, as are the lymphomas and kindred diseases. Tumors of the pleura and diaphragm receive brief attention.

The written text is compact, is conservative in tone, and makes no effort to plunge into controversial aspect. The illustrations are numerous and excellent in quality, and in their selection a fine critical sense is manifested. There is an excellent balance between gross and microscopic pathology, and the number of x-ray reproductions is not excessive. Bibliographies are adequate and up-to-date. The volume fulfills all the desiderata of an atlas of tumor pathology and is highly recommended.

Recent Advances in Nutrition Research with Emphasis on the Newer B Vitamins. Proceedings of the Scientific Sessions of the Seventh Annual Meeting, The National Vitamin Foundation, Incorporated, New York City, April 3, 1952: Nutrition Symposium Series Number 5. Edited by Robert S. Goodhart, M.D. Price, $1.50. Pp. 129, with illustrative tables and figures. The National Vitamin Foundation, Inc., New York, 1952.

Lehrbuch der Röntgendiagnostik: Teil II. Innere Organe, 7. Lieferung (Herz 2. Teil-Verdauungswege 1. Teil). By H. R. Schinz, W. E. Baensch, E. Friedl, E. Uehlinger, and with contributions from 13 other authors. Fifth edition. Price, 89 German marks. Pp. 452, with 515 illustrations and 7 tables. Georg Thieme, Diemershaldenstrasse 47, (14a) Stutgart-O; agents for U. S. A.: Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1952.

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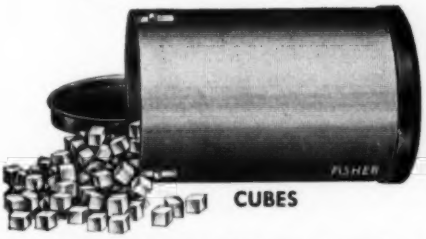

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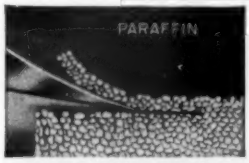

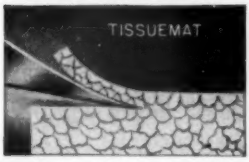

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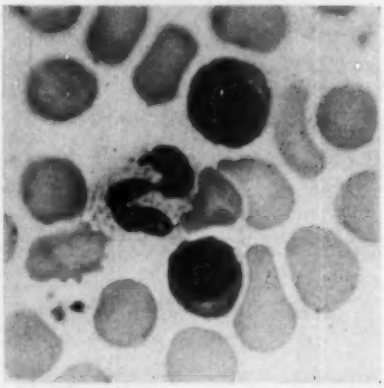

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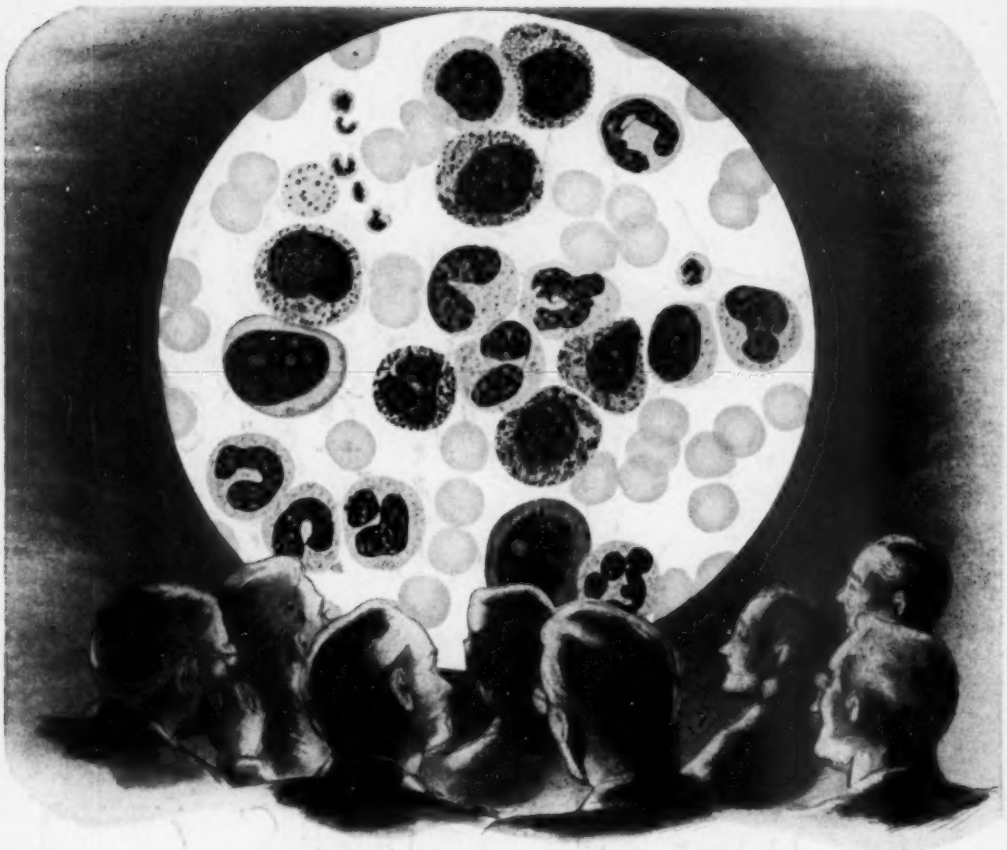

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